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Aus dem Institut für Tierernährung und Stoffwechselphysiologie
der Agrar- und Ernährungswissenschaftlichen Fakultät
der Christian-Albrechts-Universität zu Kiel

INVESTIGATIONS ON ENERGY METABOLISM OF JUVENILE TURBOT

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General Introduction

Aquaculture continues to be the fastest-growing branch producing foods from animal origin with an average annual growth rate of 5.3% in volume terms from 2003-2008 and it is expected that aquaculture will meet more than 50% of global food fish consumption by 2012 (FAO, 2010a,b). Total aquaculture production in 2008 (exclusive aquatic plants) is divided in 60% from freshwater and 40% from brackish and marine waters. Thereby carps are the most cultured fish species by volume (39%) followed by other freshwater fish (especially tilapias) and salmonids (FAO 2010b). Regarding marine fish the flatfish production increased significantly (from 26.3 mt in 2000 to 148.8 mt in 2008), whereat turbot (*Psetta maxima*), bastard halibut (*Paralichthys olivaceus*) and tongue sole (*Cynoglossus semilaevis*) represent the major species concerned (FAO, 2010a). In Europe the production of turbot, a species of high commercial value, rapidly developed over the last decade and is highest among flatfishes (Brown, 2002; Cerda et al., 2010; FAO, 2005-2012; Person Le-Ruyet, 2002). Turbot aquaculture commenced in the 1970s in Scotland (UK). Being a species originally native to Europe it is mainly farmed in countries of the European Union (especially Spain, France and Portugal), but has also been introduced to other regions (FAO, 2005-2012). In China, for example, aquaculture of turbot has reached an annual level of 50000-60000 tonnes in recent years, which is about seven times the total culture production in Europe (FAO, 2010a). Turbot are either reared in on-shore tanks using flow-through systems (the most common technique for that species) or flat-bottomed off-shore cages (FAO, 2005-2012; Person Le-Ruyet, 2002). However, in land-based farms recirculation systems are now developing rapidly (Person Le-Ruyet, 2002). Commercial turbot feed prices amounted 900 €/tonnes (2003) and the production cost for on-growing fish is about 5-6 €/kg in tanks and 5 €/kg in cages (FAO, 2005-2012). Considering an expected increase in turbot production accompanied with decreasing market prices (Person Le-Ruyet, 2002), it is of highest relevance to improve the production efficiency of turbot farming to maintain an adequate profit margin. Feed efficiency is a crucial factor, because feeds represent a minimum of 17% of the total production costs (Person Le-Ruyet, 2002). In addition to the energy required for maintenance the feed must supply the precursors for growth and consequently the energy necessary for the synthesis and deposition of body protein and lipid. Therefore, information about the maintenance energy requirement and the efficiency of energy utilization for growth is important for a growth-related feeding and a corresponding diet formulation to improve productivity and profitability of aquaculture (Lupatsch, 2009). The latter author noted that proper feed management is also crucial with regard to environmental aspects, since feed that is neither consumed nor available to the fish will be lost to the aquatic environment.

The study of the balance among dietary energy intake, expenditure and gain is called nutritional energetics or bioenergetics (Bureau et al., 2002). The motivation of studies on bioenergetics of animals is to provide the physiological framework for the research of relationships between feeding rates and growth rates of fish subjected to different environmental conditions (Jobling, 1994). Thus, results from bioenergetics can help to predict growth and contribute to the development of feeding systems as well as to diet formulation and evaluation. It is a convention to express energy requirements as the amount of digestible energy per kg body weight (BW) or metabolic body weight (MBW) per day for different types of physical activity, metabolic processes or growth (Glencross, 2008; Hatlen et al., 2007; Helland et al., 2010; Lupatsch et al., 2003; Ohta & Watanabe, 1996; Watanabe et al., 2000a,b). Results of energy requirements are highly dependent on the specific conditions in the study (body weight, sex, activity and physiological state of fish; environment; intake and nutritional value of feed). Therefore, energy requirements are calculated for defined levels of performance (e.g. maintenance, growth or swimming activity) according to factorial approaches (Bureau et al., 2002).

Energy flow in the fish is commonly presented by nutritionists based on the the energy partition scheme and nomenclature (Fig. 0-1). Thereby the digestible energy (DE) is defined as energy intake minus the energy losses by feces (e.g., undigested feed, epithelial cells, parts of the microflora inhabiting the digestive tract) representing a better estimate of „available“ energy to the animal than gross energy (GE) of feeds and ingredients (Cho & Kaushik, 1990). Fecal energy usually ranges between 15-30% of ingested energy for fish fed practical diets and is a significant loss of energy (NRC, 2011). Different factors such as feed composition and processing, feeding rate, water temperature, salinity, fish size and feeding frequency are known to affect fecal losses, whereat the composition of the diet is the dominant factor (Jobling, 1994; Krogdahl et al., 2004; NRC, 2011; Ringø, 1991; Storebakken et al., 1998). Determining digestibility of feed in animals requires collection of the fecal material. Because total collection of feces in aquatic animals is difficult indirect methods are widely used in most species of farmed fish (NRC, 2011). This method relies on the collection of a representative sample of feces that is free of uneaten feed particles and the use of a nontoxic, inert, indigestible digestion marker (e.g., chromic oxide or titanium dioxide) added to the feed (NRC, 2011). Digestibility of nutrients and energy can be estimated from the relative enrichment of the marker in the feces compared to the concentration in the feed (NRC, 2011). Therefore, the apparent digestibility (AD) of nutrients and energy in feeds can be calculated using the following equation:

Apparent digestibility = $1- [(C_i \text{ in feed} / C_i \text{ in feces}) \times (C_n \text{ of feces} / C_n \text{ of feed})]$

where C_i is the concentration of the marker and C_n the concentration of nutrients or energy. Fecal collection with the indirect method can be done in an active (e.g., by manual stripping, dissection) or passive (e.g., by settling, siphoning, netting) manner.

Fish excrete products of nutrient catabolism, mainly ammonia, via their gills and the urine. The excretion of ammonia and other types of combustible materials, such as urea, creatinine, glucose, amino acids, trimethylamine (TMA) and trimethylamine oxide (TMAO) results in energy losses that must be accounted in the energy budget. Substracting these non-fecal losses from DE results in an estimate of the metabolizable energy (ME) value of the diet. Nitrogenous wastes represent the bulk of non-fecal energy losses of fish (NRC, 2011). Approximately 85% of nitrogenous wastes excreted by fish, including turbot, is contributed by ammonia, whereas urea usually contributes less than 15% (Dosdat et al., 1995b; Kaushik & Cowey, 1991). To quantify the non-fecal energy losses the monitoring of nitrogen (N) excretion in the water of the rearing system is a commonly used approach (Bureau et al., 2002). Kaushik (1980) and Dosdat et al. (1995a) determined N excretion rates in fish using an autoanalyzer. This method allows continuous monitoring of ammonia and urea N excretion under normal physiological conditions (Kaushik et al., 1982). Furthermore, Dosdat et al. (1995a) showed that using pooled samples for analysis yields similar results than continuous sampling.

Because direct measurement of branchial and urinary N excretion is very difficult, the use of an indirect method has been recommended as an alternative (Cho & Kaushik, 1985). Using this approach, the sum of branchial and urinary N excretion is estimated by the difference between digestible N intake and retained N. Thereby the energy loss associated with N excretion can be calculated using the energetic equivalents of 24.9 kJ g^{-1} ammonia N and 23.1 kJ g^{-1} urea N (Elliott & Davison, 1975). Based on the assumption that 15% of N is excreted as urea and 85% as ammonia (Dosdat et al., 1995b) the energetic equivalent of 24.6 kJ g^{-1} non-fecal N can be calculated. Cho & Kaushik (1990) proposed a value of 24.9 kJ g^{-1} non-fecal N excreted by fish under normal conditions due to the predominantly amount of ammonia and small energetic differences between urea N and ammonia N. Estimates of non-fecal losses are variable, but their contribution to the energy budget of fish is commonly in the range of 3-6% of DE or ME intake (Bureau et al., 2002; Kaushik, 1998; Kaushik & Médale, 1994).

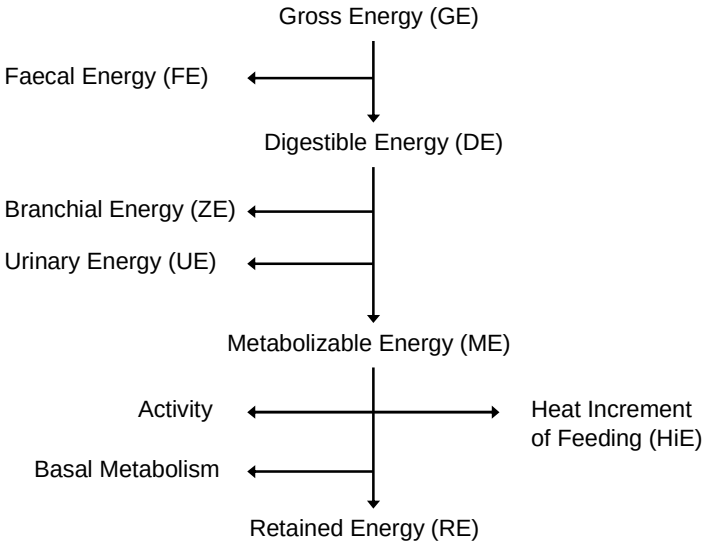

Fig. 0-1. Schematic representation of the energy flow in the fish modified after NRC (1981)

Heat is released by animals as a consequence of ATP-generation and -hydrolysis related to the metabolic transformation of dietary substrates into tissue components, due to tissue turnover as well as physical activity (Bureau et al., 2002; NRC, 2011). Total heat losses of an animal are also commonly denoted as „metabolic rate“ (Kleiber, 1975), which actually represents a much broader term. Hence, the metabolic rate is an indication of the intensity of ongoing metabolic reactions and varies dependent on the qualitative and quantitative intakes of energy and nutrient and the activity of the animal (Bureau et al., 2002; NRC, 2011). Referring to the aspects mentioned above the energy balance can be determined either by measuring the heat production or by determination of the change in total body energy content by weight and whole-body chemical analysis. Heat production can be measured by direct and indirect calorimetry. Direct calorimetry measures the heat dissipated by an animal (conduction, convection or radiant), whereas indirect calorimetry generally using the measurement of respiration (oxygen consumption, carbon dioxide production) and include the monitoring of N excretion by the animal (Bureau et al., 2002). Van Ginneken et al. (1997) showed that results of the mean heat production were comparable when using either direct or indirect calorimetry. For bioenergetic studies in fish the indirect calorimetric method is considered to be reliable and additionally more practical and less expensive than direct calorimetry (Bureau et al., 2002). In the majority of studies with fish, carbon dioxide production is ignored and only oxygen consumption is measured (Bureau et al., 2002). The latter authors noted that the amount of heat produced for each liter of oxygen (oxycalorific coefficient) used show small differences (< 10%) between oxidized lipid, carbohydrate and protein. Because a large proportion of the heat produced by fish is derived from the catabolism of amino acids and lipids as well as considering that fish are mainly ammoniotelic, the value of 19.4 kJ L^{-1} oxygen (13.6 kJ g^{-1} oxygen) was

commonly used to estimate heat production of fish (Cho & Kaushik, 1990; Elliott & Davison, 1975; Hatlen et al., 2007; Helland et al., 2010). It must be noted that a complete description of the energy balance of the fish additionally requires the mesurement of food intake together with fecal and non-fecal losses to allow the energy partitioning on the basis of DE or ME intake (Bureau et al., 2002). For practical purposes it is not always feasible to measure heat losses because of the complexity and cost of fish respirometry (Bureau et al., 2002). Therefore, a simple method based on comparative slaughter data can be applied (Blaxter, 1967; Cho & Kaushik, 1985). Using this method, at the beginning (reference group) and at the end (experimental groups) of the experiment the heats of combustion of the whole bodies of sampled fish are determined and the retained energy (RE) calculated by difference. Heat production is estimated by the difference between ME intake and RE. The minimum rate of metabolic activity needed to sustain the structure and function of the body tissues (e.g., circulation of the blood, repair and replacement of cells, membrane transport of ions, muscle tonus) is commonly denoted as basal metabolism (Bureau et al., 2002; NRC, 2011; Jobling, 1994). Feed intake increases the metabolic rate due to extra energy required for ingestion, digestion and utilization of dietary components. This increase is termed „heat increment of feeding“ (HiE) or „Specific Dynamic Action“ (SDA) (Bureau et al., 2002; McCue, 2006). Due to higher muscle tone physical activity increases metabolic rate, as well. The latter three components of animal metabolism lead to the release of energy by heat and consequently being not available for growth. To achieve a meaningful assessment of basal metabolism it is necessary to measure heat production free of any influencing factors, e.g., changes in environmental temperature, spontaneous activity and SDA (NRC, 2011). However, what is usually measured in fish studies under aquaculture conditions is the „fasting metabolic rate“ considering resting fish in a post-absorptive condition and including spontaneous activity (Jobling, 1994; NRC, 2011). Determination of oxygen consumption of free-swimming fish fasted for three to seven days is the most common approach for estimating fasting metabolic rate (Kaushik & Médale, 1994). In contrast, the term „routine metabolic rate“ is stated when defining metabolic rate of fed fish at normal activity without stress (Jobling, 1994).

Maintenance energy requirement and basal metabolism are two closely related but distinct terms (NRC, 2011). The maintenance energy requirements for DE and ME (DE_m; ME_m) are generally defined as the amount of DE or ME required to maintain zero energy balance (RE = 0). This amount of energy is the basal metabolism plus the heat increment associated with the absorption and utilization of nutrients from feed to prevent energy losses during fasting (Bureau et al., 2002). The most commonly used method for estimating DE_m or ME_m based on feeding fish at different levels accompanied by the determination of the energy balance and using regression analysis of RE as a function of DE or ME intake (Bureau et al., 2002). Thereby DE_m and ME_m are represented by

the x-intercepts and the efficiencies of DE and ME for growth ($k_{g\ (DE)}$; $k_{g\ (ME)}$) are defined by the slopes of the lines. The "factoring" of the requirements for maintenance and growth with this method, termed "The factorial approach", was explicitly reviewed by Lupatsch (2009). $K_{g\ (DE)}$ as well as $k_{g\ (ME)}$ seems to be constant and independent of fish weight, feeding level and species, but DE_m and ME_m expressed per metabolic BW are species specific (Lupatsch et al., 2003; Lupatsch, 2009). However, Imsland & Jonassen (2001) showed that there are also differences in energy utilization between distinct strains of one species. Furthermore, several studies reported an influence on energy utilization when fish meal was replaced by plant protein sources (Burel et al., 2000; Kissil et al., 2000; Morales et al., 1994). Nevertheless, the factorial approach has successfully been applied in several fish species (Azevedo et al., 1998, 2005; Booth et al., 2010; Bureau et al., 2006; Glencross, 2008; Hatlen et al., 2007; Helland et al., 2010; Lupatsch et al., 2003; Rodehutscord & Pfeffer, 1999; Watanabe et al., 2000a, 2000b) and allows to calculate the energy budget that essentially quantifies the energy the fish needs to achieve their anticipated growth (Lupatsch, 2009).

Growth in teleost fish can be affected by ecological abiotic factors whereat salinity is a typical feature regarding the aquatic environment. Many fish species, freshwater as well as seawater, have been investigated and numerous studies show that salinity clearly influences growth in fish (Boeuf & Payan, 2001). Fish are required to expend a certain amount of energy in order to meet the metabolic costs of ionic and osmotic regulation. Consequently, it has been hypothesized that if the external environment were manipulated to ensure the reduction of the metabolic costs of iono- and osmoregulation to a minimum, growth and feed utilization of fish is improved. Metabolic costs would be expected to be minimized when fish are held in iso-ionic and iso-osmotic media (Boeuf & Payan, 2001; Jobling, 1994). There are conflicting results in the literature (Morgan & Iwama, 1991) regarding the effects of an isotonic salinity on the metabolic rate and growth of fish in general as well as in turbot in particular. On the one hand, routine metabolic rate in turbot was observed to be minimal at salinities between 8-10 g L^{-1} (Waller, 1992; Gaumet et al., 1995) which classifies turbot among fish, such as rainbow trout and tilapia, with a minimal energetical level in isotonic conditions (Nordlie et al., 1991). On the other hand, Tang et al. (2006) showed a similar metabolic rate at different salinities after 48 h of adaption. Furthermore, juvenile turbot showing best growth and feed conversion efficiency at intermediate salinities (15-20 g L^{-1}), especially between 16-21 °C (Beouf et al., 1999; Gaumet et al., 1995; Imsland et al., 2001). Recent experiments indicate that the energetic cost of iono- and osmoregulation in fish remains under debate, ranging from 10 to > 50% of total energy budget (Boeuf & Payan, 2001). It has to be considered that any attempt to quantify this cost is probably affected by other factors (e.g., feed intake, hormonal changes affecting

metabolic processes, activity, feed digestibility) which respond to changes in salinity as well (Boeuf & Payan, 2001; Morgan & Iwama, 1991).

Conventional bioenergetic models (e.g., the factorial approach) are widely used to investigate the nutrient and energy utilization by fish (Azevedo et al., 1998, 2005; Booth et al., 2010; Bureau et al., 2006; Glencross, 2008; Hatlen et al., 2007; Helland et al., 2010; Lupatsch et al., 2003; Rodehutscord & Pfeffer, 1999), but their limitations need to be recognized. In general, considering only the sum of nutrients solely on the basis of their energy value does not allow a complete evaluation of the effect of chemical composition of the feed and efficiency of use of specific nutrients (NRC, 2011). One of the practical limitations of conventional bioenergetic models is that they assume that energy is allocated in a hierarchical fashion and that growth is the surplus of energy after all other components of the energy budget have been satisfied (Elliot & Hurley, 1999). Models based on bioenergetic principles assume that growth and feed efficiency will be nil when animals are fed a maintenance ration (RE = 0). That assumption has been proven inaccurate in fish, as well as in other animals, where positive weight gain was still observed even though animals were fed on a maintenance ration of a nutritionally adequate diet (Bureau et al., 2006). Live weight gain is mainly (but not solely) driven by protein deposition due to the related association of water (Dumas et al., 2007; Shearer, 1994), and lipids can be mobilized to support protein deposition (Bureau et al., 2006). Conventional bioenergetic models commonly rely on estimates of the cost of growth calculated empirically based on statistical interpretation of experimental data, such as direct relationships between daily lysine or energy intake and daily growth (Birkett & de Lange, 2001; NRC, 2011). Application of such empirical models is limited to animal, environmental and management conditions, i.e., how much of the energy cost is truly due to “biological inefficiencies” or simply due to the fact that animals are fed “inadequate” diets is not known. Furthermore, these empirical estimates cannot be legitimately extrapolated to conditions beyond which data are collected (France & Thornley, 1984). The latter models offer little insight into the mechanistic biological principles (metabolism of absorbed nutrients) as well, of which the measured performance is a consequence (Birkett & de Lange, 2001).

In contrast to the empirical approach, highly complex mechanistic biochemical models have been developed to simulate nutrient metabolism at the level of individual tissues, using differential equations to represent (non-causally) relationships between the various metabolite flow rates (Birkett & de Lange, 2001; NRC, 2011). In practice mechanistic models are not easy to use effectively to predict whole animal response, because the construction of these models requires adequate knowledge of the system; especially on the organizational level (cellular, whole animal, animal groups) the model is primarily designed to represent, and relies on sufficient and accurate

data to quantify the perceived system (Baldwin, 1995; Birkett & de Lange, 2001). The process of parameterization can be a major bottleneck in the development and application of complex mechanistic models (Dumas et al., 2010). Consequently, all biochemical models have been developed with some degree of simplification of metabolic pathways, have included numerous assumptions and have been generally driven by more or less transparent partitioning rules (NRC, 2011). However, biochemical models can provide unnecessary and undesirable details, which make them often more complex than what is required to present growth at the whole animal level (Birkett & de Lange, 2001). Furthermore, the large number of parameters that must be estimated, in some instances without solid experimental observations, limits the predictive flexibility and applicability to describe nutrient utilization by fish as influenced by a wide range of conditions (differences in feed composition, environmental conditions, husbandry practices, life stages and genetic background of animals) encountered in fish culture, a weakness shared with the empirical approach (Birkett & de Lange, 2001). Despite its limitations, the factorial approach is still common in bioenergetics (Dumas et al., 2008).

Referring to the different aspects mentioned in the previous sections, with special focus on turbot aquaculture, the present experiments intended to determine the maintenance energy requirements (DE_m and ME_m) and the efficiencies of energy utilization for growth ($k_{g\ (DE)}$ and $k_{g\ (ME)}$) as well as the effects of biotic (fish strain, dietary protein source) and abiotic (salinity) factors on the latter parameters and total energy budget in order to provide basic data applicable for the development of adequate feeding charts in turbot aquaculture. Two methodical different experiments were realized. A 1st one using a common growth trial in combination with the comparative slaughter technique and considering the influence of turbot strain as well as the partial replacement of fish meal by wheat gluten (*chapter 1*). The 2nd experiment applied respirometry to examine if the results are similar to the 1st experiment and respirometry is suitable to determine DE_m, ME_m, $k_{g\ (DE)}$ and $k_{g\ (ME)}$ in turbot (*chapter 2*). Additionally, the 2nd experiment also considered the influence of salinity on the energy metabolism.

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Chapter 1

ENERGY REQUIREMENT FOR MAINTENANCE AND EFFICIENCY OF ENERGY UTILIZATION FOR GROWTH IN JUVENILE TURBOT (*PSETTA MAXIMA*, L.): THE EFFECT OF STRAIN AND REPLACEMENT OF DIETARY FISH MEAL BY WHEAT GLUTEN

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Abstract

Restricted ration level experiments were realized to determine the gross, digestible and metabilizable energy requirements for maintenance (GE_m; DE_m; ME_m) and the efficiencies of energy utilization for growth ($k_{g\ (GE)}$; $k_{g\ (DE)}$; $k_{g\ (ME)}$) in juvenile turbot. The effects of the fish strain (Denmark, DK; Iceland, IS) as well as the partial replacement of fish meal by wheat gluten in the diet were examined as possible factors affecting the energy metabolism. Turbot (initial body weight 49g) were reared in a recirculation aquaculture system supplied with seawater at a temperature of 17 ± 0.6 °C and 26 ± 1.2 ppt salinity for 67 days. Two diets, differing in wheat gluten content (80 g or 330 g kg^{-1} diet) were fed at six increasing feeding levels, from near maintenance to *ad libitum* levels, once a day per hand. Chemical composition and energy content of body weight gain were determined by using the comparative slaughter technique. Linear regression analyses were applied to obtain GE_m, DE_m, ME_m, $k_{g\ (GE)}$, $k_{g\ (DE)}$ and $k_{g\ (ME)}$.

GE_m, DE_m and ME_m were 21.6-29.8, 17.0-23.5 and 15.5-21.4 kJ $kg^{-0.8}d^{-1}$, respectively, and $k_{g\ (GE)}$, $k_{g\ (DE)}$ and $k_{g\ (ME)}$ were 0.46-0.49, 0.59-0.64 and 0.63-0.68, respectively. Values for GE_m, DE_m and ME_m as well as for $k_{g\ (GE)}$, $k_{g\ (DE)}$ and $k_{g\ (ME)}$ in turbot from IS were significantly higher than in fish from DK, but without any effect on energy retention at high feeding levels. Turbot receiving the diet where fish meal was partially replaced by wheat gluten showed higher GE_m, DE_m and ME_m as well. No effect on energy retention at high feeding levels was observed again. It is concluded that differences in energy metabolism between strains might be considered when selecting turbot for aquaculture production.

1. Introduction

Information on energy requirements and efficiencies of energy utilization in cultured fish species allow for scientifically based strategies for feed formulation and feeding regimes to ensure efficient production (Bureau et al., 2002). In Europe, the production of turbot, a species of high commercial value, rapidly developed over the last decade and is highest among flatfishes (Cerda et al., 2010; FAO, 2011; Person Le-Ruyet, 2002). Nevertheless, detailed information on energy requirement and energy utilization of turbot is limited and mainly derived from experiments with indirect calorimetry (Brown et al., 1984; Mallekh & Lagardere, 2002; Waller, 1992). One method to quantify and separate the nutritional requirements for maintenance and growth in aquaculture, noted as factorial approach, was described by several authors (Huisman, 1976; Lupatsch, 2009): Fish are fed restricted ration levels (RRL) and energy retention (RE) is regressed against digestible energy (DE) or metabolizable energy (ME) intake, where DE or ME requirements for maintenance (DE_m, ME_m) are defined as intakes at RE = 0 and the efficiencies of energy utilization for growth above maintenance ($k_{g\ (DE)}$; $k_{g\ (ME)}$) as the slopes. This method was applied in several fish species e.g. Atlantic salmon (*Salmo salar*), gilthead seabream (*Gilthead seabream*), European seabass (*Dicentrarchus labrax*), white grouper (*Epinephelus aeneus*), rainbow trout (*Oncorhynchus mykiss*), Atlantic cod (*Gadus morhua*), barramundi (*Lates calcarifer*) and yellowtail kingfish (*Seriola quinqueradiata*) (Azevedo et al., 1998, 2005; Booth et al., 2010; Bureau et al., 2006; Glencross, 2008; Hatlen et al., 2007; Helland et al., 2010; Lupatsch et al., 2003; Rodehutscord & Pfeffer, 1999; Watanabe et al., 2000a, 2000b).

Imsland & Jonassen (2001) indicated that there are differences in energy utilization and growth performance between various strains of wild turbot stocks. The latter study suggested that the observed differences were caused by the adaption to different environmental conditions (e.g. compensation of a shorter growing season by higher growth performance).

Furthermore, energy utilization in fish can be affected when replacing fish meal by plant protein sources (Burel et al., 2000; Kissil et al., 2000; Morales et al., 1994). Wheat gluten (WG) is commonly used in fish diets due to high protein contents, low amounts of fibre and anti-nutritional factors as well as a high apparent digestibility (Bonaldo et al., 2011; NRC, 2011; Robaina et al., 1999). It is included in turbot diets up to levels of 23% without negative effects on growth performance (Fournier et al., 2004). WG is known to have a lower biological value of protein than fish meal and lysine represents the first limiting amino acid (Kaushik & Seiliez, 2010). Therefore, replacing fish meal by WG in fish diets reduce lysine intake (Helland & Grisdale-Helland, 2006). It was shown that a deficient dietary lysine intake at an adequate energy supply could change energy partitioning of weight gain in fish due to a decrease in protein and an increase in lipid deposition

(Cheng et al., 2003; Rodehutscord et al., 1997). Investigations in poultry by Nieto et al. (1995) showed that $k_{g\ (ME)}$ increased as protein to lipid ratio of weight gain decreased. The authors suggested that this effect might be attributable to less energy requirements and higher RE associated with lipid deposition than protein deposition. Additionally, they reported that both $k_{g\ (ME)}$ and ME_m decreased inversely to the dietary protein quality.

The present study aimed to determine the maintenance energy requirement for gross energy (GE_m), DE_m and ME_m as well as the corresponding efficiencies of energy utilization for growth above maintenance $k_{g\ (GE)}$, $k_{g\ (DE)}$ and $k_{g\ (ME)}$ in juvenile turbot using the factorial approach and to test whether the strain of fish and the partially replacement of fish meal by wheat gluten affects energy metabolism.

2. Material and methods

2.1 Fish

Juvenile turbot (*Psetta maxima*, L.) of two different strains (Denmark; Iceland) were used for the experiment. Danish individuals (DK) were received from the hatchery Maximus A/S (Bedsted, Denmark) and Icelandic ones (IS) from Marine Research Institute (MRI, Grindavik, Iceland). DK descended from a domestic Norwegian broodstock, whereas IS from an Icelandic broodstock (wild and domestic). Fish were transferred to the rearing facilities of Gesellschaft für Marine Aquakultur, GMA (Büsum, Germany) on 29th september (DK) and 29th october (IS) in 2009, respectively.

2.2 Experimental Diets

Two diets were formulated: a fish meal based diet A and a fish meal reduced diet B, which partially replaced fish meal (255 g kg^{-1} diet) with wheat gluten. Diet A and B were formulated to be similar in gross energy (GE), nitrogen (N) and lipid content. Referring to Peres & Oliva-Teles (2008) the two diets were formulated to meet the indispensable amino acid (IAA) requirements of turbot except diet B where the lysine content in crude protein was slightly lower (4.5 g 16 g^{-1} N) than recommended (5.0 g 16 g^{-1} N). Titanium dioxide (10 g kg^{-1}) was added to the diets as an inert marker to determine the digestibility of the diets. Detailed information about feed components and chemical composition of the experimental diets are given in Table 1-1. Diets were pelleted (4 mm diameter; pellet press 14-175, AMANDUS KAHL GmbH & Co. KG, Hamburg, Germany).

Table 1-1:

Feed components and chemical composition of the experimental diets (g or MJ kg^{-1})

	Diet A	Diet B
Feed components		
Herring fish meal LT[a]	748	493
Fish oil[a]	75	90
Wheat starch (pregelatinized)[b]	80	70
Wheat gluten[b]	80	330
Vitamin premix[c]	3.5	3.5
Mineral premix[d]	3.5	3.5
Titanium dioxide (TiO_2)[e]	10	10
Chemical composition		
Dry matter (DM)	941	940
In DM		
Crude protein (N × 6.25)	617	643
Indispensable amino acids[f]		
Arg	38	34
Lys	40	29
His	14	14
Ile	25	25
Leu	44	45
Val	30	29
Met	16	14
Phe	25	24
Thr	25	22
Trp[h]	5.4	5.6
Crude lipid	172	169
Crude ash	146	108
Gross energy (GE)	22.5	23.1
Digestible energy (DE)[g]	17.7	18.2
DCP:DE (g MJ^{-1})	30.3	31.9
Ca	36	25
P	22	15
TiO_2[i]	10	10

[a] Vereinigte Fischmehlwerke Cuxhaven GmbH & Co. KG, Cuxhaven, Germany;
[fish meal made from herring offal: DM, Crude protein, crude lipid, crude ash = 948; 724, 127, 114 g kg^{-1}, respectively; LT = low-temperature cooking and drying (80-90 °C)]

[b] Kröner Stärke – H. Kröner GmbH, Ibbenbüren, Germany; (Wheat gluten: DM, Crude protein = 920, 780 g kg^{-1}, respectively)

[c] MicroMineral (supply per kilogram of diet): 1.4 mg $CoSO_4$; 8.75 mg $MnSO_4$; 1.75 mg CaI_2; 8.75 mg $CuSO_4$; 0.09 mg Se

[d] Vitamin Standard (supply per kilogram of diet): 3500 I.U. vitamin A; 700 I.U. vitamin D3; 140 mg vitamin E (alpha-Tocopherol-acetate); 14 mg vitamin K3 (Menadione); 14 mg vitamin B1; 28 mg vitamin B2; 14 mg vitamin B6; 0.03 mg vitamin B12; 210 mg vitamin C (Monophosphate); 28 mg vitamin B3; 140 mg vitamin B7; 5.60 mg Folic Acid; 0.35 mg Biotin; 140 mg Inositol; 700 mg Betain-anhydrate; 98 mg $ZnSO_4$; 140 mg Etoxyquin
(contents of vitamins, minerals and additives as specified by the manufacturer)

[e] Kronos Titan GmbH & Co.oHG, Nordenham, Germany

[f] Hydrated (non-protein-bound) form of amino acids

[g] Determined in the present study

DCP = digestible crude protein (determined in the present study)

[h] Estimated from Trp content of feed components: fishmeal (6.5 g kg^{-1}; analyzed by VDLUFA ITL GmbH, Kiel, Germany), wheat gluten (7.2 g kg^{-1}; analyzed by Evonik Degussa GmbH, Hanau, Germany); Trp was determined by HPLC with fluorescence detection (extinction 280 nm, emission 356 nm), after alkaline hydrolysis with barium hydroxide octahydrate for 20 h at 110°C (Commission Directive 45/EC, 2000)

[i] Calculated by: $TiO_2 = Ti \times 1.67$

2.3 Experimental Conditions

The experiment was carried out in 54 tanks (145 L, bottom surface: 0.27 m^2) connected to a closed recirculation aquaculture system (RAS) equipped with sedimentation tank (~ 300 L), skimmer, moving bed biofilter (~ 648 L), UV-sterilizer, sump (~ 2064 L) and pumps at Gesellschaft für Marine Aquakultur (Büsum, Germany). The RRL design including six feeding levels (I-VI), which corresponded to a daily feed supply of 0.3, 0.6, 0.9, 1.2 and 1.5% of body weight (BW) was applied. Additional experimental groups fed to apparent satiation were used to verify if maximum feed intake has been covered by the highest defined feeding levels. While diet B was offered to DK only, diet A was given to DK and IS. To adjust the quantity of supplied feed to the increasing BW, fish of each tank were group weighed every 20 days. Each diet and strain treatment was fed in three replications per feeding level once a day per hand. All uneaten feed was siphoned out of the tanks about 10 minutes after feeding, subsequently dried at 103 °C and weighed. Additionally, the weights of recovered uneaten pellets were corrected for soluble losses incurring between feeding and collection to make an accurate feed intake assessment. Soluble losses were determined prior start of the trial by soaking several pellets in a beaker filled with tank water for 10 min, then sampled and dried like the remaining pellets in the fish tanks. Two weeks before the experimental period 648 juvenile turbot (39-45 g; DK: n = 432, IS: n = 216) were randomly distributed to the experimental groups (12 fish per tank) and transferred to the tanks for acclimatisation. Additionally, 12 fish of each strain, to be used as reference group for body composition analysis, were randomly selected and transferred to collateral tanks as well. The two weeks of acclimatisation until the start of the trial experimental and reference groups were fed 1.0% of BW with a commercial feed (Aller 505, Emsland - AllerAqua GmbH, Golßen, Germany; 5 mm diameter). At the beginning of the experiment fish had a mean initial BW of 49 ± 3 g and were then reared for 67 days at 17 ± 0.6 °C and 26 ± 1.2 ppt salinity. This is within the range for optimal growth of juvenile turbot (Burel et al., 1996; Imsland et al., 2001c). Initial stocking density was 1.1 kg m^{-2} and not above 4.0 kg m^{-2} at the

end of experiment, which is similar to values reported for optimal growth of juvenile turbot (Irwin et al., 1999; Ma et al., 2006). Dissolved oxygen, temperature (OxyGuard-Handy Polaris 2, OxyGuard International A/S, Birkerod, Denmark), salinity (HI 96822 digital seawater refractometer, HANNA instruments Inc., Woonsocket-RI-USA), total ammonia nitrogen (TAN) and nitrite nitrogen (colorimetric test kit - Microquant; Merck KgaA, Darmstadt, Germany) were measured daily. Dissolved oxygen was maintained above 7.0 mg L^{-1}. The mean TAN was 0.1 ± 0.1 mg L^{-1} and never exceeded 0.2 mg L^{-1}. Mean nitrite nitrogen (NO_2-N) was 1.2 ± 0.3 mg L^{-1} with a maximum of 1.5 mg L^{-1}. TAN and NO_2-N values observed in this study were lower than the concentrations reported in the literature for growth limitation (Alderson, 1979; Bianchini et al., 1996; Person Le-Ruyet et al., 1997; Person Le-Ruyet, 2002; Rasmussen & Korsgaard, 1996). The photoperiod was maintained on a 12 h L:12 h D cycle.

2.4 Measurements and sampling

Fish were weighed individually at the beginning and at the end of the experiment to calculate the average growth performance of each experimental group. Fish starved one day prior to weighing. The comparative slaughter technique was applied to determine retained nutrients and energy: At the beginning of the experiment 10 individuals (two samplings of 5 fish) of each reference group and at the end three individuals per experimental group were killed by an overdose of MS-222 (200 mg L^{-1}), chopped and stored at -18 °C. Fish of the final samplings represented the average fish of the respective tank (difference in weight profile does not exceed 10, 20 and 25 g for feeding levels I-III, IV and V, respectively). Additionally, seven fish of each feeding level were killed at the end of experiment to record the liver weights as well as for sampling blood and muscle tissue (data not included in the present study). The remaining turbot from feeding levels III and IV on diet A (DK and IS pooled) and diet B (DK) were used for determination of nutrient and energy digestibility of the respective diets. For this, three replicates for diet A and two replicates for diet B were used. All replicates (n = 20 fish per replicate) had a similar total body weight (average per fish 143 g). Thereby fish of 56 – 160 g BW were used to cover the range between the different feeding levels of the experiment. At each diet fish were fed two weeks once daily at 1.0% of BW. Approximately 12 h after the last feeding the content of the posterior intestine (from the ileocaecal valve to the anus) was carefully sampled by dissection once fish were killed by an overdose of MS-222 (200 mg L^{-1}). No abrasion of intestinal walls was performed to minimize the contamination of digesta with epithelial cells. Due to limited quantities to do accurate chemical analyses the sampled intestinal contents were pooled for each diet and stored in a plastic container at -18 °C. Afterwards the fish as

well as the intestinal contents was freeze-dried. Prior to chemical analyses freeze-dried fish of each reference and tank samplings were separately pooled and homogenized by centrifugal mill (ZM 100, Retsch GmbH &Co.KG, Haan, Germany), whereas intestinal samples by using mortar and pestle.

2.5 Chemical Analysis

According to Naumann & Bassler (1976) diets, fish and intestinal contents were analyzed in duplicate (diets, intestinal samples) and triplicate (fish) for dry matter (DM), crude ash (CA), crude lipid (CL), crude fibre (CF) and gross energy (GE; adiabatic bomb calorimeter C200, IKA-Werke GmbH & Co.KG, Staufen, Germany). CL analysis was done with (diets) or without (fish, intestinal content) HCl hydrolysis, because lower lipid extraction was observed when applying HCl hydrolysis in sampled fish and intestinal contents. Crude protein (CP, N × 6.25) was determined using the Dumas combustion principle (True Spec N, LECO Corporation, St. Joseph, MI, USA). TiO_2 in diets and intestinal content was determined according to Brandt & Allam (1987): TiO_2 was solubilized by the Kjeldahl procedure for 3 h in 0.96 g g^{-1} sulphuric acid, then 0.35 g g^{-1} hydrogen peroxide (H_2O_2) was added to the filtered TiO_2 solution to form a yellow complex and color intensity measured in a spectrophotometer (visible spectrophotometer 6300, Barloworld Scientific Ltd. T/As Jenway, Dunmow, Essex, U.K.) at 405 nm. IAA contents (except tryptophan) of the diets were determined by ion-exchange chromatography with postcolumn derivatization with ninhydrin (Commission Directive 98/64/EC, 1998). The determination of calcium and phosphorus contents of diets and fish was performed according to VDLUFA VII 2.2.2.6 method (VDLUFA, 2008).

2.6 Definitions, Calculations and Statistics

Geometric mean body weight (GMBW), g: $(BW_0 \times BW_1)^{0.5}$, where BW_0 and BW_1 are initial and final mean BW per tank, respectively.

Metabolic body weight (MBW), $kg^{0.8}$: $(GMBW/1000)^{0.8}$. Despite indications that a metabolic weight exponent of 0.7 provides a better approximation to a weight-dependent energy expenditure in flatfish (Waller, 1992) the weights of turbot in the present study were scaled to a metabolic weight exponent for energy of 0.8 due to a better comparison with other fish species. The latter exponent is suggested to be a common value in different fish species (Lupatsch et al., 2003), although the majority of results range between 0.65 and 0.90 (Jobling, 1994). Nevertheless, the

metabolic weight exponent of 0.8 was used in several studies (Booth et al., 2010; Glencross, 2008; Hatlen et al., 2007; Helland et al., 2010; Huisman, 1976; Pirozzi et al., 2010).

Specific growth rate (SGR), % per day: $100 \times (\ln BW_1 - \ln BW_0)/days_{exp}$, where $days_{exp}$ are the experimental days.

Feed efficiency ratio (FER): $(BW_1-BW_0)/FI_{tot}$, where FI_{tot} is total feed DM intake during the experimental period.

Hepatosomatic index (HSI), %: $100 \times$ final liver weight/BW_1.

Apparent digestibility coefficients (ADC), %: $100 \times [1-(\%\ feed_{TiO2}/\%\ feces_{TiO2}) \times (\%\ feces_n/\%\ feed_n)]$, where subscript n stands for a specific nutrient or energy.

To calculate energy of digestible protein intake (DEI_p) and retained protein energy (RE_p) the energetic equivalent of 23.6 kJ g^{-1} protein (Blaxter, 1989) was used.

Referring to Helland et al. (2010) the non-faecal N excretion was calculated as the difference between digestible N intake and N deposition. The energy loss associated with this excretion was calculated using the energetic equivalents of 24.9 kJ g^{-1} ammonia N and 23.1 kJ g^{-1} urea N (Elliott and Davison, 1975). Assuming 15% of N is excreted as urea and 85% as ammonia (Dosdat et al., 1995) results in 24.6 kJ g^{-1} non-faecal N.

Gross energy intake (GEI), digestible energy intake (DEI), metabolizable energy intake (MEI) and retained energy (RE) at feeding levels I-V were subjected to linear regression analysis for determination of GE_m, DE_m, ME_m, $k_{g\ (GE)}$, $k_{g\ (DE)}$ and $k_{g\ (ME)}$ according to the following linear regression equations:

$$RE\ (kJ\ kg^{-0.8}\ d^{-1}) = a + k_{g\ (GE)} \times GEI\ (kJ\ kg^{-0.8}\ d^{-1})$$

$$RE\ (kJ\ kg^{-0.8}\ d^{-1}) = a + k_{g\ (DE)} \times DEI\ (kJ\ kg^{-0.8}\ d^{-1})$$

$$RE\ (kJ\ kg^{-0.8}\ d^{-1}) = a + k_{g\ (ME)} \times MEI\ (kJ\ kg^{-0.8}\ d^{-1})$$

Energy intakes at RE = 0 are defined as the maintenance requirements, and the efficiencies of energy utilization for growth above maintenance are given by the slopes. However, this statistical approach does not provide the standard errors for the estimates of the energy maintenance requirements, which are necessary for testing the significance of differences. Therefore, GE_m, DE_m and ME_m were alternatively determined by the following equations, where energy intake is taken as dependent and RE as independent variable:

$GEI\ (kJ\ kg^{-0.8}\ d^{-1}) = GE_m + 1/k_{g\ (GE)} \times RE\ (kJ\ kg^{-0.8}\ d^{-1})$

$DEI\ (kJ\ kg^{-0.8}\ d^{-1}) = DE_m + 1/k_{g\ (DE)} \times RE\ (kJ\ kg^{-0.8}\ d^{-1})$

$MEI\ (kJ\ kg^{-0.8}\ d^{-1}) = ME_m + 1/k_{g\ (ME)} \times RE\ (kJ\ kg^{-0.8}\ d^{-1})$

Here, GE_m, DE_m and ME_m are represented by the y-intercepts and the slopes $1/k_{g\ (GE)}$, $1/k_{g\ (DE)}$ and $1/k_{g\ (ME)}$ - the reciprocals of the k_g -values - describe the costs for GE, DE or ME (kJ) per unit of RE (kJ), respectively. It must be noted that GE_m, DE_m and ME_m -values determined by extrapolation at RE = 0 include also the energetic requirements for spontaneous activity.

All the data were checked for homogeneity of variance using box plots and residual plots. Two-sided t-test was used to compare slopes and y-intercepts of the linear regression equations. Results of growth and energy budget parameters were tested by ANOVA applying linear models (y ~ diet + feeding level + diet × feeding level) followed by multiple comparisons between diet A and diet B as well as DK and IS for each of the five feeding levels. Additionally, all-pair comparisons of the five feeding levels were performed within DK (diet B), DK (diet A) and IS (diet A), respectively. For data showing heterogeneity of variance the respective statistical procedures were applied according to Hasler & Hothorn (2008). Differences between experimental groups were considered to be significant at the $p < 0.05$ level. All statistical data analyses were performed using R-Software version 2.11.1 (R Development Core Team, 2010). Results are presented as means ± SD. Fish at the *ad libitum* levels showed high variation in feed intake. Therefore, resting time and consequently dissolution of uneaten pellets were higher than in the other feeding levels. Thus, the determined feed intake is considered to be inaccurate and consequently these groups were excluded from all statistical tests and regressions.

3. Results

3.1 Digestibility, feed intake, feed efficiency, growth and body composition

No fish died during the experiment. The estimates of the apparent digestibilities of the two different diets are shown in Table 1-2. Despite the lack of error terms, the estimated ADC of protein appeared to be slightly lower in the fish meal based diet A than in the fish meal reduced diet B. The reverse effect could be observed in lipid digestibility, whereas estimated ADC-values of energy, organic matter and dry matter were similar between diets. Feed intake varied between 1.50 – 1.53 g DM $kg^{-0.8}\ d^{-1}$ at the lowest and 6.95 – 7.37 g DM $kg^{-0.8}\ d^{-1}$ at the highest feeding levels (Table 1-3).

Table 1-2:

Apparent digestibility[a] (%) of the experimental diets

	Diet A	Diet B
Dry matter	74.8	73.2
Organic matter	81.0	81.0
Crude protein	87.0	90.3
Crude lipid	90.7	89.0
Energy	78.6	78.8

[a] Calculated by using one pooled sample of intestinal content from turbot fed diet A (n = 60 fish, Danish and Icelandic turbot pooled) and diet B (n = 40 fish)

FER, SGR, HSI (Table 1-3) and final body composition (Table 1-4) did not differ between diets and between strains at the same feeding level. DM, CL and energy content of final whole body increase with increasing feeding level ($p < 0.001$), whereas CA content showing the reverse relationship ($p < 0.001$). Final body protein concentration seems to be independent from feeding level. SGR, HSI ($p < 0.001$) and FER ($p < 0.05$) showed also higher values as feed intake increased with the exception of FER in turbot (DK) fed diet A.

Table 1-3:

Body weight, feed intake, feed efficiency, growth and HSI of juvenile turbot from two strains fed diet A or B for 67 days

Treatment	BW [g]		Feed intake	SGR	FER	HSI
Feeding level	initial	final	[g $kg^{-0.8}d^{-1}$, DM]	[% BW d^{-1}]	[g gain g^{-1} feed]	[%]
Diet B (DK)						
I	48.1 ± 0.4	59.5 ± 0.5^{A}	1.53 ± 0.01^{A}	0.32 ± 0.02^{A}	1.18 ± 0.07^{A}	0.8 ± 0.2^{A}
II	48.7 ± 0.5	82.7 ± 4.4^{A}	3.05 ± 0.05^{B}	0.79 ± 0.08^{B}	1.60 ± 0.29^{B}	1.1 ± 0.2^{AB}
III	49.2 ± 1.0	118.0 ± 3.0^{C}	4.75 ± 0.02^{Cy}	1.30 ± 0.01^{C}	1.69 ± 0.04^{B}	1.5 ± 0.3^{BC}
IV	48.8 ± 0.7	141.4 ± 4.8^{D}	6.35 ± 0.07^{D}	1.59 ± 0.03^{D}	1.59 ± 0.05^{B}	1.8 ± 0.3^{CD}
V	49.3 ± 0.7	160.6 ± 3.5^{D}	7.19 ± 0.09^{E}	1.76 ± 0.04^{E}	1.60 ± 0.02^{B}	2.3 ± 0.4^{D}
Diet A (DK)						
I	47.3 ± 0.9	58.2 ± 1.9^{A}	1.50 ± 0.01^{A}	0.31 ± 0.04^{A}	1.31 ± 0.33	0.8 ± 0.1^{Av}
II	49.4 ± 1.2	85.5 ± 1.9^{B}	3.07 ± 0.06^{B}	0.82 ± 0.03^{B}	1.49 ± 0.18	1.1 ± 0.1^{AB}
III	48.6 ± 1.2	113.7 ± 1.9^{C}	4.63 ± 0.03^{Cxv}	1.27 ± 0.02^{C}	1.70 ± 0.05	1.4 ± 0.2^{BC}
IV	47.9 ± 1.0	136.7 ± 3.0^{D}	6.42 ± 0.11^{D}	1.56 ± 0.02^{D}	1.54 ± 0.02	1.6 ± 0.1^{C}
V	48.0 ± 0.9	149.6 ± 5.8^{D}	7.37 ± 0.12^{E}	1.70 ± 0.03^{D}	1.46 ± 0.08	1.8 ± 0.3^{C}
Diet A (IS)						
I	48.0 ± 0.8	57.5 ± 1.9^{A}	1.52 ± 0.03^{A}	0.27 ± 0.05^{A}	1.02 ± 0.15	1.0 ± 0.2^{Aw}
II	48.6 ± 0.8	80.1 ± 4.0^{AB}	3.04 ± 0.04^{B}	0.75 ± 0.06^{B}	1.41 ± 0.12	1.2 ± 0.2^{AB}
III	48.4 ± 1.7	101.8 ± 10.1^{ABC}	4.69 ± 0.02^{Cw}	1.11 ± 0.10^{BC}	1.43 ± 0.14	1.5 ± 0.1^{BC}
IV	47.8 ± 0.6	137.0 ± 1.8^{C}	6.32 ± 0.08^{DC}	1.57 ± 0.01^{C}	1.57 ± 0.03	1.6 ± 0.3^{AC}
V	48.8 ± 1.1	151.6 ± 16.5^{BC}	6.95 ± 0.44^{C}	1.68 ± 0.17^{BC}	1.56 ± 0.11	1.7 ± 0.2^{C}

Values are mean ± SD of triplicate feeding groups; within each column values with different superscripts are significantly different ($p < 0.05$); capital letters showing contrasts between feeding levels within strains and diets; x, y and v, w - letters showing contrasts between diets and strains at one feeding level, respectively; DK, Danish strain; IS, Icelandic strain; DM, dry matter; BW, body weight; SGR, specific growth rate; FER, feed efficiency ratio; HSI, hepatosomatic index (n = 7 fish per feeding level)

Table 1-4:

Initial and final body composition (g 100 g^{-1}or kJ g^{-1} wet weight) of juvenile turbot from two strains fed diet A or B for 67 days

Treatment Feeding level	Dry matter	Crude protein	Crude lipid	Crude ash	Energy
initial (DK)[a]	22.4 ± 0.5	14.7 ± 0.1	3.9 ± 0.6	3.8 ± 0.0	4.9 ± 0.2
initial (IS)[a]	22.4 ± 0.1	14.3 ± 0.4	4.2 ± 0.1	3.6 ± 0.1	5.0 ± 0.1
Diet B (DK)					
I	21.1 ± 0.5^{A}	14.5 ± 0.3	2.8 ± 0.4^{A}	4.1 ± 0.1^{A}	4.3 ± 0.3^{A}
II	21.8 ± 0.2^{A}	14.9 ± 0.1	3.6 ± 0.4^{AB}	3.9 ± 0.2^{A}	4.8 ± 0.1^{AB}
III	22.2 ± 0.3^{AB}	14.6 ± 0.2	4.6 ± 0.2^{BC}	3.2 ± 0.1^{B}	4.9 ± 0.3^{AB}
IV	22.5 ± 0.4^{AB}	14.9 ± 0.3	4.6 ± 0.3^{B}	3.3 ± 0.0^{B}	5.2 ± 0.1^{BC}
V	23.9 ± 0.5^{B}	15.4 ± 0.3	5.6 ± 0.2^{C}	3.4 ± 0.2^{B}	5.6 ± 0.2^{C}
Diet A (DK)					
I	21.3 ± 1.5^{A}	14.6 ± 0.9^{AB}	2.8 ± 0.2^{A}	4.3 ± 0.3^{A}	4.4 ± 0.3^{A}
II	22.3 ± 0.4^{AB}	14.7 ± 0.5^{AB}	3.9 ± 0.2^{B}	3.8 ± 0.2^{AB}	4.9 ± 0.1^{AB}
III	22.5 ± 0.4^{AB}	14.4 ± 0.5^{A}	4.1 ± 0.3^{B}	3.6 ± 0.2^{BC}	5.2 ± 0.1^{BC}
IV	23.4 ± 1.1^{B}	15.1 ± 0.3^{AB}	4.9 ± 0.8^{BC}	3.5 ± 0.3^{BC}	5.4 ± 0.4^{BC}
V	23.9 ± 0.4^{B}	15.7 ± 0.5^{B}	5.2 ± 0.4^{C}	3.2 ± 0.1^{C}	5.7 ± 0.1^{C}
Diet A (IS)					
I	21.2 ± 0.9^{A}	14.6 ± 0.5	2.9 ± 0.5^{A}	4.1 ± 0.3^{A}	4.4 ± 0.3^{A}
II	21.9 ± 0.5^{AB}	15.1 ± 0.0	3.4 ± 0.4^{AB}	3.9 ± 0.2^{A}	4.9 ± 0.3^{AB}
III	22.6 ± 0.2^{ABC}	15.1 ± 0.3	4.0 ± 0.4^{BC}	3.7 ± 0.2^{AB}	5.1 ± 0.1^{BC}
IV	23.2 ± 0.4^{BC}	14.9 ± 0.1	4.7 ± 0.3^{CD}	3.4 ± 0.2^{B}	5.4 ± 0.1^{BC}
V	24.1 ± 1.1^{C}	15.3 ± 0.6	5.4 ± 0.4^{D}	3.4 ± 0.2^{B}	5.5 ± 0.2^{C}

Values are mean ± SD of triplicate feeding groups; within each column values with different superscript letters are significantly different ($p < 0.05$); capital letters showing contrasts between feeding levels within strains and diets; DK, Danish strain; IS, Icelandic strain

[a] Body weights, g: 52.7 ± 1.0 (DK), 52.0 ± 0.0 g (IS); body composition was not different between strains ($p \geq 0.05$); values are mean ± SD of two samples (5 fish each)

3.2 Energy budget

The energy budgets of the experimental groups are shown in Table 1-5. Assuming that digestibility was not affected by feeding level 21.4% and 21.2% of GEI were calculated to be lost as fecal energy for diet A and B, respectively. Energy excretion via ammonia and urea significantly increased as digestible protein intake increased with feeding level. In average, energy loss by ammonia and urea excretion amounted 7.3% of DEI (5.7% of GEI), resulting in ME-values representing approximately 92.9% of DE (73.0% of GEI).

At level V 39.6 - 40.7% of GEI was converted into body energy, whereas only 7.3-11.6% was retained in level I due to the higher proportion of GE_m. RE and RE_p were similar between the two strains and diets, respectively. Depending on the feeding level 63.2 - 80.2% of RE were deposited as protein. This proportion decreased when feeding level increased ($p < 0.05$). In all treatments RE at level I was lower than RE_p, indicating depletion of body lipid reserves. Despite of the latter aspect RE were positive in all feeding levels.

Table 1-5:

Energy budget and protein utilization of juvenile turbot from two strains fed diet A or B for 67 days

Treatment Feeding level	GEI	DEI	MEI	DEI_p (kJ $kg^{-0.8}d^{-1}$)	E_N-loss	RE	RE_p	GE_m/GEI	RE/GEI [%]	RE_p/RE
Diet B (DK)										
I	35.3 ± 0.2^{Ay}	27.9 ± 0.1^{Ay}	25.3 ± 0.2^{A}	20.9 ± 0.1^{Ay}	2.5 ± 0.0^{A}	3.2 ± 2.9^{A}	5.7 ± 0.4^{A}	82.2 ± 0.4^{Ay}	9.1 ± 8.2	- [a]
II	70.4 ± 1.2^{B}	55.5 ± 0.9^{B}	51.3 ± 0.7^{B}	41.5 ± 0.7^{B}	4.2 ± 0.3^{AB}	20.9 ± 3.2^{B}	16.6 ± 1.5^{B}	41.2 ± 0.7^{By}	29.7 ± 4.9	80.0 ± 5.8
III	109.8 ± 0.5^{Cy}	86.6 ± 0.4^{Cy}	80.3 ± 0.2^{Cy}	65.1 ± 0.3^{Cy}	6.2 ± 0.2^{By}	39.4 ± 3.4^{C}	27.7 ± 0.7^{C}	26.5 ± 0.1^{Cy}	35.9 ± 2.9	70.8 ± 8.0
IV	146.8 ± 1.6^{D}	115.8 ± 1.2^{D}	107.2 ± 1.0^{D}	87.0 ± 0.9^{Dy}	8.6 ± 0.3^{C}	53.9 ± 2.7^{CD}	35.7 ± 0.9^{D}	19.8 ± 0.2^{Dy}	36.7 ± 2.1	66.3 ± 1.8
V	166.3 ± 2.2^{E}	131.1 ± 1.7^{E}	121.7 ± 1.7^{E}	98.5 ± 1.3^{E}	9.4 ± 0.2^{C}	67.2 ± 2.9^{D}	42.5 ± 1.7^{D}	17.5 ± 0.2^{Ey}	40.5 ± 2.0	63.2 ± 2.9
Diet A (DK)										
I	33.6 ± 0.3^{Ax}	26.4 ± 0.2^{Ax}	24.2 ± 0.4^{A}	18.9 ± 0.2^{Ax}	2.2 ± 0.2^{A}	3.9 ± 1.7^{A}	5.8 ± 1.3^{A}	64.2 ± 0.6^{Avx}	11.6 ± 4.9^{A}	- [a]
II	69.0 ± 1.4^{B}	54.3 ± 1.1^{B}	50.5 ± 0.8^{B}	38.9 ± 0.8^{B}	3.7 ± 0.3^{B}	23.8 ± 0.4^{B}	16.7 ± 1.1^{B}	31.3 ± 0.6^{Bvx}	34.5 ± 1.2^{AB}	70.1 ± 3.7
III	103.9 ± 0.6^{Cx}	81.7 ± 0.5^{Cx}	76.3 ± 0.6^{Cx}	58.5 ± 0.3^{Cx}	5.4 ± 0.2^{Cx}	41.6 ± 2.4^{C}	26.1 ± 1.4^{C}	20.8 ± 0.1^{Cvx}	40.0 ± 2.1^{AB}	62.9 ± 3.9
IV	144.3 ± 2.4^{D}	113.4 ± 1.9^{D}	105.8 ± 1.9^{D}	81.3 ± 1.4^{Dx}	7.6 ± 0.0^{D}	55.7 ± 5.8^{BCD}	35.7 ± 1.1^{D}	15.0 ± 0.3^{Dvx}	38.6 ± 3.5^{B}	64.4 ± 5.1
V	165.6 ± 2.7^{E}	130.2 ± 2.1^{E}	121.5 ± 2.1^{E}	93.3 ± 1.5^{E}	8.7 ± 0.1^{E}	65.6 ± 1.8^{D}	41.5 ± 1.4^{D}	13.0 ± 0.2^{Evx}	39.6 ± 1.0^{B}	63.2 ± 1.6
Diet A (IS)										
I	34.1 ± 0.8^{A}	26.8 ± 0.6^{A}	24.5 ± 0.7^{A}	19.2 ± 0.4^{A}	2.3 ± 0.2^{A}	2.5 ± 3.0^{A}	5.6 ± 1.3^{A}	87.7 ± 2.0^{Aw}	7.3 ± 8.8	- [a]
II	68.2 ± 1.0^{B}	53.6 ± 0.8^{B}	50.0 ± 0.6^{B}	38.4 ± 0.5^{B}	3.6 ± 0.3^{B}	21.1 ± 4.4^{AB}	16.6 ± 1.3^{B}	43.8 ± 0.6^{Bw}	31.0 ± 6.6	80.2 ± 13.0
III	105.3 ± 0.4^{C}	82.8 ± 0.3^{C}	77.1 ± 0.5^{C}	59.4 ± 0.2^{C}	5.8 ± 0.3^{C}	35.4 ± 5.2^{BC}	24.8 ± 1.8^{BC}	28.3 ± 0.1^{Cw}	33.6 ± 4.9	70.5 ± 5.4
IV	142.0 ± 1.9^{D}	111.7 ± 1.5^{D}	104.3 ± 1.3^{D}	80.0 ± 1.0^{D}	7.4 ± 0.2^{D}	55.9 ± 0.9^{CD}	35.7 ± 0.1^{D}	12.0 ± 0.3^{Dw}	39.4 ± 1.2	63.8 ± 1.2
V	156.2 ± 10.0^{CD}	122.8 ± 7.9^{CD}	114.9 ± 7.3^{CD}	88.0 ± 5.6^{CD}	7.9 ± 0.8^{BCD}	63.5 ± 4.1^{D}	40.4 ± 4.4^{BCD}	19.2 ± 1.2^{Dw}	40.7 ± 2.1	63.6 ± 2.9

Values are mean ± SD of triplicate feeding groups; within each column values with different superscript letters are significantly different ($p < 0.05$);

capital letters defining contrasts between feeding levels within diets and strains; x,y - letters defining contrasts between diets at one feeding level; v,w - letters defining contrasts between strains at one feeding level; DK, Danish strain; IS, Icelandic strain; GEI, gross energy intake, DEI, digestible energy intake; MEI, metabolizable energy intake; DEI_p, energy intake from digestible protein; E_N, energy of non-faecal N (ammonia + urea); RE, retained energy; RE_p, retained energy (protein); GE_m, maintenance energy requirement of GE determined in the present study (29.0, 21.6 and 29.8 kJ $kg^{-0.8}$ d^{-1} for diet B (DK), diet A (DK) and diet A (IS), respectively).

* Not given due to body fat loss

3.3 Maintenance energy requirement and efficiency of energy utilization for growth

The relationships between RE (kJ $kg^{-0.8}d^{-1}$) and GEI (kJ $kg^{-0.8}d^{-1}$), RE and DEI (kJ $kg^{-0.8}d^{-1}$) as well as RE and MEI (kJ $kg^{-0.8}d^{-1}$) are described by the following regression equations and Fig. 1-1, Fig. 1-2, and Fig. 1-3, respectively.

Diet B (DK): $RE = -13.1 (\pm 2.0) + 0.473 (\pm 0.017) \times GEI$ (1)
($R^2 = 0.98$)

Diet A (DK): $RE = -9.2 (\pm 1.9) + 0.459 (\pm 0.017) \times GEI$ (2)
($R^2 = 0.98$)

Diet A (IS): $RE = -13.8 (\pm 2.6) + 0.490 (\pm 0.020) \times GEI$ (3)
($R^2 = 0.98$)

Diet B (DK): $RE = -13.1 (\pm 2.0) + 0.600 (\pm 0.022) \times DEI$ (4)
($R^2 = 0.98$)

Diet A (DK): $RE = -9.3 (\pm 1.9) + 0.583 (\pm 0.021) \times DEI$ (5)
($R^2 = 0.98$)

Diet A (IS): $RE = -13.8 (\pm 2.3) + 0.623 (\pm 0.026) \times DEI$ (6)
($R^2 = 0.98$)

Diet B (DK) $RE = -12.8 (\pm 2.0) + 0.644 (\pm 0.023) \times MEI$ (7)
($R^2 = 0.98$)

Diet A (DK) $RE = -9.0 (\pm 1.9) + 0.623 (\pm 0.022) \times MEI$ (8)
($R^2 = 0.98$)

Diet A (IS) $RE = -13.5 (\pm 2.1) + 0.664 (\pm 0.026) \times MEI$ (9)
($R^2 = 0.98$)

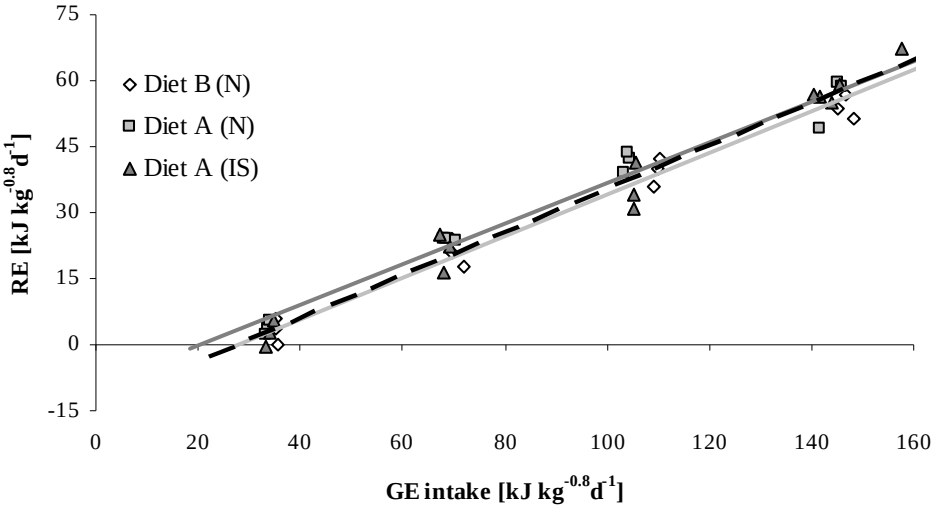

Fig. 1-1. Daily energy retention per kilogram metabolic body weight (RE) of juvenile turbot fed increasing levels of gross energy (GE) for diets A and B and for fish from two strains [Denmark, DK; Iceland, IS]. Each data point represents the mean of triplicates; equations (1)-(3) are given in the text.

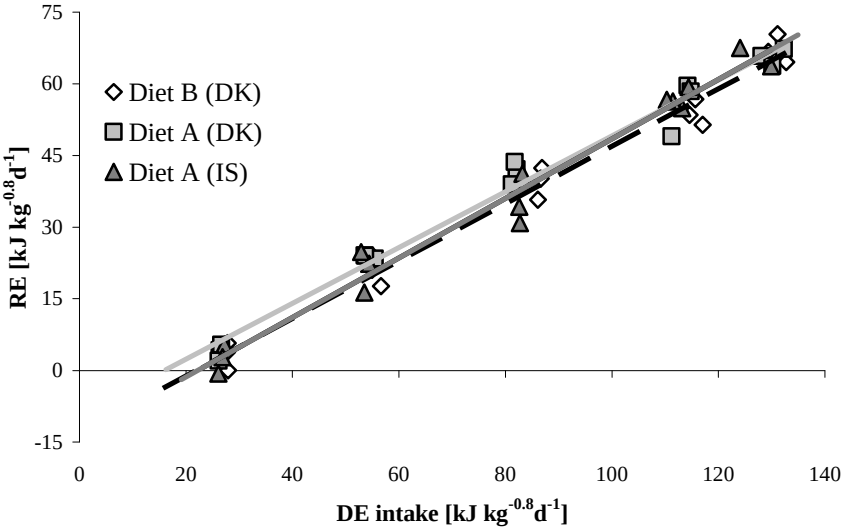

Fig. 1-2. Daily energy retention per kilogram metabolic body weight (RE) of juvenile turbot fed increasing levels of digestible energy (DE) for diets A and B and for fish from two strains [Denmark, DK; Iceland, IS]. Each data point represents the mean of triplicates; equations (4)-(6) are given in the text.

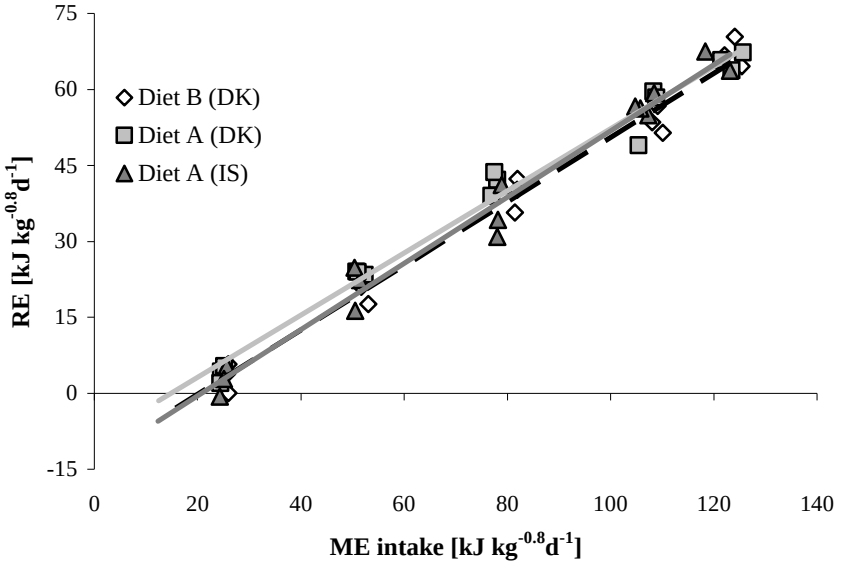

Fig. 1-3. Daily energy retention per kilogram metabolic body weight (RE) of juvenile turbot fed increasing levels of metabolizable energy (ME) for diets A and B and for fish from two strains [Denmark, DK; Iceland, IS]. Each data point represents the mean of triplicates; equations (7)-(9) are given in the text.

Results of linear regression of the relationship between GEI (y) and RE (x), DEI (y) and RE as well as MEI (y) and RE are summarized in Table 1-6. The determined $k_{g\ (GE)}$-values of 0.481 ($1/k_{g\ (GE)}$ = 2.08), 0.467 ($1/k_{g\ (GE)}$ = 2.14) and 0.500 ($1/k_{g\ (GE)}$ = 2.00) for diet B (DK), diet A (DK) and diet A (IS), respectively, were similar to corresponding $k_{g\ (GE)}$-values of linear regression equations (1)-(3). Thus, results of statistical comparisons between $1/k_g$-values and between k_g-values were similar, too. $K_{g\ (GE)}$-values differ significantly between diets and strains ($p < 0.02$). Furthermore, GE_m was lower ($p < 0.05$) in turbot (DK) fed diet A than fed diet B and higher ($p < 0.05$) in IS than DK (diet A). Similar statistical correlations were obtained when considering the relationship between DEI and RE as well as MEI and RE. However, there were no differences in ME_m, $k_{g\ (DE)}$ and $k_{g\ (ME)}$ between the two diets ($p = 0.06$, 0.14 and 0.12, respectively).

Table 1-6:

Parameters of linear regression equations (n=15)[a] between GEI, DEI, MEI and RE, respectively.

Treatment	GE_m	$1/k_{g\,(GE)}$	DE_m	$1/k_{g\,(DE)}$	ME_m	$1/k_{g\,(ME)}$
DK - Diet B	29.0 (± 3.3)[a]	2.08 (± 0.08)[a]	22.9 (± 2.6)[a]	1.64 (± 0.06)	20.8 (± 2.4)	1.53 (± 0.05)
DK – Diet A	21.6 (± 3.5)[bx]	2.14 (± 0.08)[bx]	17.0 (± 2.7)[bx]	1.69 (± 0.06)[x]	15.5 (± 2.5)[x]	1.58 (± 0.06) [x]
IS - Diet A	29.8 (± 3.5)[y]	2.00 (± 0.08)[y]	23.5 (± 2.8)[y]	1.57 (± 0.07)[y]	21.4 (± 2.5)[y]	1.48 (± 0.06) [y]

[a] $R^2 = 0.98$, $p < 0.001$, similar for all treatments

$GEI = GE_m + 1/k_{g\,(GE)} \times RE$; $DEI = DE_m + 1/k_{g\,(DE)} \times RE$; $MEI = ME_m + 1/k_{g\,(ME)} \times RE$

(GEI, DEI, MEI, RE, GE_m, DE_m, ME_m expressed as kJ $kg^{-0.8}$ d^{-1});

Values (mean ± SE) in the same column with different superscripts are significantly different ($p < 0.05$); a, b - letters = contrasts between diets; x, y - letters = contrasts between strains; GEI, gross energy intake; DEI, digestible energy intake; MEI, metabolizable energy intake; RE, retained energy; GE_m, maintenance requirement of gross energy; DE_m, maintenance requirement of digestible energy; ME_m, maintenance requirement of metabolizable energy; $1/k_{g\,(GE)}$, energetic costs of GE (kJ) per unit RE (kJ); $1/k_{g\,(DE)}$, energetic costs of DE (kJ) per unit RE; $1/k_{g\,(ME)}$, energetic costs of ME (kJ) per unit RE.

4. Discussion

4.1 Digestibility and growth performance

Dietary composition is of major importance for the digestibility of the diet (Jobling, 1994). Non-digested DM in the present study contributed a relatively large amount (26%). This value is based on the ADC determinations at one feeding level only (1% BW). Studies on the effect of feeding level on ADC have produced contradictionary results, ranging from those that have found a decrease of ADC-values with increasing feeding level (Helland et al., 2010; Henken et al., 1985; Luo et al., 2006; Xie et al., 1997) to other studies that have found increased ADC-values (Han et al., 2004; Storebakken et al., 1991). The observed effects on ADC of nutrients and especially energy in the latter studies were of lower magnitude when considering the range of feeding levels used in the present study (except for DM differences are commonly less than 2.5%). Furthermore, results of Fernández et al. (1998) showed that feeding level has no effect on final ADC and support the hypothesis that for highly digestible diets, an increase in feeding level would have no or only small effects on overall digestibility, but that a progressive decrease in digestibility with increasing feeding level could be expected when diets of low quality are fed to the fish. Both diets in the present study were formulated based on feed ingredients known to be well digested by fish (NRC, 2011) as well as included only low levels of moderately digestible gelatinized starch. Commonly, most studies show that feeding levels have no impact on digestibility of nutrients (assuming that feed intake is not below levels needed to support reasonable growth rates) and intestinal absorptive

capacity is not likely to be a limiting factor for nutrient digestibility (NRC, 2011). Referring to the previous aspects the use of the same ADC-values of nutrients and energy for all feeding levels is considered to be acceptable. Due to the low cohesion accompanied with dissolution in the water body it was very difficult to collect feces from turbot. The collection of feces by the sedimentation method or siphoning was less successful as well as can result in overestimated ADC-values due to leaching (Jobling, 1994, NRC, 2011). Furthermore, the latter authors noted that obtaining digesta prior to its natural voidance as feces by stripping or dissection may result in the collection of an unknown amount of incompletely digested material probably causing underestimated ADC-values. Despite this disadvantage stripping or dissection has been used in several studies with flatfish (Berge et al., 1991; Dias et al., 2010; Grisdale-Helland & Helland, 1998; Hatlen et al., 2005). Turbot appeared to be able to control the release of digesta from the rectal pouch, which complicated feces stripping. Therefore, it was decided to use the method of dissection the hind gut combined with a marker to obtain accurate samples of digesta. Similar difficulties in the collection of feces (digesta) were reported in other flatfish (Dias et al., 2010; Grisdale-Helland & Helland, 1998). To ensure a complete digestion of the diets feces in the present study were sampled 12 h after the last meal referring to the procedure in other studies with turbot and flatfish (Bonaldo et al., 2011; Dias et al., 2010) except using stripping. Due to low amounts of digesta sampled in the hind gut, it was impossible to realize chemical analyses within each replicate. Thus, we decided to pool the samples from all replicates within each diet. Therefore, statistical analysis was not possible resulting in estimated ADC-values. However, the observed ADC-values can be considered as representative as ADC-values of dietary nutrients and energy are in the range of values determined by other studies with turbot (Fournier et al., 2004; Oliva-Teles et al., 1999; Peres & Oliva-Teles, 2005; Regost et al., 2001, 2003) and Atlantic halibut (*Hippoglossus hippoglossus*) (Berge & Storebakken, 1991; Grisdale-Helland & Helland, 1998) at similar dietary composition. Variations of ADC-values between studies may be caused by using different feces collecting methods.

Due to pooling fish from DK and IS related to the ADC determination in the growth trial the effect of fish strain on ADC of nutrients and energy could not be investigated. But it can be assumed that no differences in ADC (as well as non-fecal N excretion) between strains were present, because the absence of differences in FER, SGR (Table 1-3), protein and lipid retention ($p > 0.24$, 0.28, 0.82 and 0.32, respectively). Therefore, pooling fish from DK and IS for feces collection should not be affected the results in the present study.

The present study indicated a tendency of a slightly better digestibility of protein when replacing fish meal by wheat gluten. Storebakken et al. (2000) showed a similar effect in Atlantic salmon. Our observations can may be related to a better protein digestibility of WG (ADC: 99-100%) than

herring fish meal (ADC: 91-96%) as observed in several fish species (NRC, 2011). Protein from bones and scales are not likely well digested (NRC, 1993) and thus reduce ADC of protein from fish meal. Several studies suggested that protein digestibility in fish meal is affected by the processing conditions, e.g. cooking and drying temperature during meal manufacture, the quality of added fish solubles as well as the subsequent meal storage, and therefore can cause variations even between batches of the same source. (Aksnes & Mundheim, 1997; Anderson et al., 1995, 1997). The previous aspects probably explain the lower ADC-values in fish meal than wheat gluten and therefore the observed lower protein digestibility in diet A in the present study.

Considering the usage of the same lipid sources in diet A and diet B and the difference determined in lipid digestibility between diets was only 1.7% a true difference probably does not exist, but actually represent the lack of precision due to no replication. The higher ADC of protein but the lower ADC of lipid in diet B than diet A resulted in absent differences in ADC of energy and organic matter between diets. The determined ADC-values in the current study resulted only from one pooled sample per diet and the observed differences in ADC of protein as well as lipid should be considered as indications to be verified in further experiments.

SGR, FER and HSI determined in the current experiments are in the line with results from other studies in turbot (Caceres- Martinez et al., 1984; Fournier et al., 2004; Oliva-Teles et al., 1999; Regost et al., 2001). The generally high performance level and the absence of any differences in growth performance and body composition between the two diets at the same feeding levels indicate that lysine supply of diet B was adequate, although not expected according to the recommendations of Peres & Oliva-Teles (2008).

4.2 Energy budget

The higher DEI_p in fish fed diet B may be a result of the slightly higher protein content and the slightly better protein digestibility. As known from other studies in various fish species (Ballestrazzi et al., 1994; Beamish & Thomas, 1984; Dosdat et al., 1995; Kaushik & Medale, 1994; Vergara et al., 1996) the higher energy losses by N excretion in diet B than in diet A can be attributed to the higher DEI_p.

Fonds et al. (1992) showed that 53-55% of MEI results in RE and 42-47% is lost as heat (ME_m + activity + heat increment of feeding) in plaice (*Pleuronectes platessa*) and flounder (*Platichthys flesus*) when fed to satiation. These values are in the line with ours at the highest feeding levels (RE/MEI = 54-55%). The ratio RE/GEI for fish fed at level V is higher than reported by Peres & Oliva-Teles (2005) for juvenile turbot (32 g initial BW, 18 °C, RE/GEI = 22-33%), but lower than

in Atlantic halibut (61 g initial BW, 12 °C; RE/GEI = 48-50%; Helland & Grisdale-Helland, 2006) fed similar diets, respectively. Differences in RE between the studies may be also depended on feed ingredients and digestibility as well as experimental conditions and investigated species. Bureau et al. (2002) noted that protein deposition has a generally high priority in fish. Therefore, the excess of retained protein energy (RE_p) compared to RE at feeding level I in all treatment groups could be attributed to either a decrease in protein turnover or preferential catabolism of other substrates like body lipid. Lipid deposition tends to increase with increasing feed (energy) supply (Jobling, 1994), indicated by the decreasing ratio RE_p/RE with increasing feeding level in the present study. Similar observations were reported by Bureau et al. (2002) and Lupatsch et al. (2003). At the highest feeding level (V), RE_p/RE ratios (63.2-63.6%) ranged between values determined in other studies with turbot (815 g BW, 17 °C, RE_p/RE = 76.6%; Regost et al., 2001) or Japanese flounder (*Paralichthys olivaceus*) (6 g BW, 18-22 °C, RE_p/RE = 77.6-83.4%; Yigit et al., 2004) and Atlantic halibut (49.4-53.4%; Helland & Grisdale-Helland, 2006).

4.3 Maintenance energy requirement and efficiency of energy utilization for growth

Although the limitations accompanied by the ADC determination should only affect the actual values of DE_m and ME_m as well as $k_{g\ (DE)}$ and $k_{g\ (ME)}$, but not the respective statistics itself, the following discussion primarily regard the results at the GE-level. However, for comparisons with other fish species results at the DE- and ME-level are considered as well.

GE_m, DE_m and ME_m of turbot in this study (extrapolated from the linear regression) are in the line with values in other flatfish like Dover sole (*Solea solea*), but considerably lower than values for other aquaculture species, e.g. European seabass, Gilthead seabream, Atlantic cod, Barramundi or Atlantic salmon (Table 1-7). This might be caused by a lower physical activity of flatfish (Fonds et al., 1992). The k_g-values are at the lower point of the range of other marine fish (Table 7). Therefore, the latter data as well as our results support the statement of Lupatsch et al. (2003) that maintenance requirement expressed per kg metabolic body weight is species specific, but contradict the position that k_g is relatively constant and independent from fish species. It has to be considered that k_g is independent from BW, temperature and feeding level, whereas GE_m, DE_m and ME_m are affected by BW and temperature (Azevedo et al., 1998; Lupatsch et al., 2003). Furthermore, significant intraspecific differences of GE_m, DE_m and ME_m between distinct strains of turbot were observed in the present study.

The distinct values of GE_m (DE_m, ME_m) and $k_{g\ (GE)}$ ($k_{g\ (DE)}$, $k_{g\ (ME)}$) between strains or diets in our study cannot be related to changes in protein and lipid deposition, because no differences have been observed ($p > 0.20$).

Breeding as well as hatchery programmes in aquaculture usually aim in production of fast growing strains of fish (Jobling, 1994). The latter author pointed out that strains known to grow most rapidly and most efficiently often show low rates of protein breakdown and turnover leading to lower costs to maintain status quo. Therefore, it can be hypothesized that the differences in energy requirements and energy utilization efficiencies between strains may be caused by the kind of hatchery selection or breeding management as suggested by Bailey and Alanärä (2006) and Imsland et al. (2001b).

Imsland et al. (2001a, 2001b) and Imsland & Jonassen (2001) reported that there are interpopulation differences in growth performance of turbot from different localities. Additionally, Imsland et al. (2000) observed differences in energy required for maintenance and spontaneous activity as well as energy conversion efficiencies in different geographic populations of juvenile halibut. Therefore, energy utilization efficiencies as well as maintenance energy requirements may vary between geographic populations of turbot as well. Results from the present study support this hypothesis. However, the genetic backgrounds of both strains of turbot in the present study were not examined and the geographic origin of the wild broodstock of the Danish fish is unknown and needs to be clarified in further experiments. Thus, the assumption of an effect of geographic origin on population differences in GE_m and $k_{g\ (GE)}$ is highly speculative. Referring to Bailey and Alanärä (2006), the differences in GE_m between fish from DK and IS may be also the result of individual social interactions and spontaneous activity, e.g. a higher feed searching behavior or a reduced activity to spare energy. Despite the differences in GE_m and $k_{g\ (GE)}$, energy retention at a high feeding level was similar between the two strains in our study. This was caused by the inverse relationship between maintenance energy requirements and the related energy efficiencies for growth, i.e. the superior $k_{g\ (GE)}$-values of fish from Iceland were compensated by higher GE_m-values. Referring to the total energy allocation the relative proportion of GE_m decrease and of RE increase with increasing feed intake. Therefore, the k_g-values become more important than GE_m when considering feed efficiency and growth at high energy intake. Despite there were no differences in RE between strains, we expect that at high feeding levels turbot (IS) will show the highest feed efficiency and growth due to a more favourable combination of GE_m and $k_{g\ (GE)}$. Further growth experiments are needed, especially with larger fish, to confirm the latter assumptions.

Table 1-7:

Maintenance energy requirements and efficiencies of energy utilization for growth in different fish species

Author	Spezies (BW)	DE_m kJ $kg^{-0.8}d^{-1}$	$k_{g\,(DE)}$	Experimental temperature
Present study[b]	Turbot (55-176 g)	17.0-23.5 21.6-29.8[a] 15.5-21.4[c]	0.59-0.64 0.47-0.50[a] 0.63-0.68[c]	17 °C
Ende et al., 2009	Dover sole (44-100 g)	29.4[a]	0.39[a]	19 °C
Lupatsch et al., 2003	European sea bass (1-400 g)	45.4	0.67	19-26 °C
	Gilthead sea bream (1-250 g)	47.9	0.69	23-24 °C
	White grouper (6-230 g)	34.1	0.69	23 °C
Hatlen et al., 2007	Atlantic cod (250 g)	42.3	0.80	10 °C
Glenncross, 2008	Barramundi			30 °C
	(15 g)	35.1	0.61	
	(410 g)	45.5	0.76	
Helland et al., 2010	Atlantic salmon (96 g)	31.5 30.6[c]	0.80 0.82[c]	10 °C

BW, body weight

DE_m, maintenance requirement of digestible energy; $k_{g\,(DE)}$, efficiency of digestible energy utilization for growth

[a] ME_m, maintenance requirement of metabolizable energy; $k_{g\,(ME)}$, efficiency of metabolizable energy utilization for growth

[b] Values represent the range of GE_m, DE_m, ME_m and $k_{g\,(GE)}$, $k_{g\,(DE)}$, $k_{g\,(ME)}$ of all treatments

[c] GE_m, maintenance requirement of gross energy; $k_{g\,(GE)}$, efficiency of gross energy utilization for growth

$K_{g\,(GE)}$ in the present study was different ($p = 0.01$) between diet A and diet B, whereas Helland & Grisdale-Helland (2006) reported no differences in $k_{g\,(GE)}$ when replacing fish meal by WG (10-30%) in diets for Atlantic halibut. In fact, the latter difference in $k_{g\,(GE)}$ between diets was really small and thus can probably considered to be neglectable from a practical point of view. No study in the literature was found, which indicates effects of replacing dietary fish meal by any plant protein source on maintenance energy requirements of fish. However, in growing chickens Nieto et al. (1995) showed that methionine supplementation to a methionine deficient diet reduced ME_m and k_g

$_{(ME)}$ without negative effects on growth and RE. This effect was assumed to be caused by a reduced muscle protein breakdown resulting in lower energetic costs for protein turnover as well as an increased and decreased efficiency of protein and lipid retention, respectively. Regarding the requirements of fish the IAA profile of WG is considered to be inferior than fish meal (Kaushik & Seiliez, 2010). Thus, the replacement of fish meal by WG might changed the IAA balance of diet B. Results of the present study were similar to the observations by Nieto et al. (1995) mentioned above, however, without using diets deficient in IAA. Whether our results were also caused by changes in body protein breakdown needs to be further investigated.

The observed differences in GE_m ($p = 0.01$) and DE_m ($p = 0.04$), but not in ME_m ($p = 0.06$) between diets can be attributed to the lower variation of the relationship between GEI and RE (DEI and RE) than MEI and RE. Due to a similar ADC of energy and E_N-loss the differing $k_{g\ (GE)}$ between diets is likely a result of a lower variation as well.

Conclusion

The estimated GE_m (DE_m, ME_m)- and $k_{g\ (GE)}$ ($k_{g\ (DE)}$, $k_{g\ (ME)}$)-values in the present study can help to estimate energy requirements of growing juvenile turbot reared in a RAS more precisely. The present study indicates differences in GE_m, DE_m, ME_m, $k_{g\ (GE)}$, $k_{g\ (DE)}$ and $k_{g\ (ME)}$ between strains of turbot, however without any significant effect on growth performance. Whether the observed differences in GE_m , DE_m or ME_m and $k_{g\ (GE)}$, $k_{g\ (DE)}$ or $k_{g\ (ME)}$ will result in different growth performance between IS and DK at different experimental conditions than in the current study, e.g. fish size, temperature range or photoperiod, needs to be investigated in further studies. It is concluded that it might be advisable to consider the strain of fish when selecting turbot used in aquaculture production.

Furthermore, it was shown that the partial replacement of fish meal by WG increased GE_m and $k_{g\ (GE)}$, DE_m and $k_{g\ (DE)}$ as well as ME_m and $k_{g\ (ME)}$. Nevertheless, WG can replace fish meal up to a total inclusion level of 330g kg^{-1} in diets for juvenile turbot without showing negative effects on growth performance and influencing RE.

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Chapter 2

INFLUENCE OF SALINITY ON ENERGY METABOLISM IN JUVENILE TURBOT (*PSETTA MAXIMA*, L.)

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Abstract

Oxygen consumption was measured for 24 h in juvenile turbot, *Psetta maxima* (L.) using flow-through respirometry to examine the influence of salinity on energy metabolism. Turbot (164 g mean initial body weight, BW) were reared at 16.5 ± 0.2 °C and three different salinities (10, 20, 30 g L^{-1}). Digestible and metabolizable energy requirements for maintenance (DE_m; ME_m) as well as the respective efficiencies of energy utilization for growth ($k_{g\ (DE)}$; $k_{g\ (ME)}$) were identified by using different feeding levels (0.3, 0.6, 0.9% BW) and applying linear regression analysis.

There is evidence that nutrient and energy digestibilities decrease with an increasing salinity. DE_m and ME_m, $k_{g\ (DE)}$ and $k_{g\ (ME)}$ were 14.9-20.2 and 13.3-18.3 kJ $kg^{-0.8}d^{-1}$, 0.82-0.87 and 0.87-0.91, respectively. Generally, no differences were observed in DE_m, ME_m, $k_{g\ (DE)}$, and $k_{g\ (ME)}$ between salinities, but it was indicated that these parameters decrease at a high salinity (30 g L^{-1}). Furthermore, turbot showed the most favourable combination of DE_m and $k_{g\ (DE)}$ (ME_m and $k_{g\ (ME)}$) at a salinity of 20 g L^{-1} as well as became inferior at 30 g L^{-1} regarding growth and energy utilization at high energy intake. However, energy requirements for iono- and osmoregulation are small.

1. Introduction

In Europe the production of turbot, *Psetta maxima* (L.), a species of high commercial value, rapidly developed over the last decade and is highest among flatfish (Person Le-Ruyet, 2002; Cerda et al., 2010; FAO, 2005-2012). Considering an expected increase in turbot production will be accompanied with decreasing market prices (Person Le-Ruyet, 2002), it is of highest relevance to improve the production efficiency of turbot farming to maintain an adequate profit margin. Feeding efficiency is a crucial factor, because feeds represent a minimum of 17% of the total production costs (Person Le-Ruyet, 2002). Quantifying the maintenance energy requirement and the efficiency of energy utilization for growth is a prerequisite to derive an adequate feed composition and supply, so that least-cost formulation can optimize the balance between nutrient requirements and the cost of feed (Lupatsch, 2009). Proper feed management is also crucial with regard to environmental aspects, since feed that is neither consumed nor available to the fish will be lost to the aquatic environment (Lupatsch, 2009). Growth of fish generally depends on energy supply considering factors like feed intake, feed composition, feed digestibility and non-fecal energy losses as well as the energy expenditures related to maintenance requirements, several metabolic processes (digestion, excretion), activity and adaption to rearing conditions. The digestible energy (DE) and metabolizable energy (ME) requirements for maintenance (DE_m, ME_m) and the efficiencies of energy utilization for growth ($k_{g\ (DE)}$; $k_{g\ (ME)}$) in fish can be quantified by using different feeding levels and linear regression analysis of the relationship between DE (ME) intake and the retained energy (RE). The “factoring” of the requirements for maintenance and growth with this method, termed “The factorial approach”, was explicitly reviewed by Lupatsch (2009) and had successfully been applied in several fish species e.g. Atlantic salmon, *Salmo salar* (L.); gilthead seabream, *Sparus aurata* (L.); European seabass, *Dicentrarchus labrax* (L.); white grouper, *Epinephelus aeneus* (Saint-Hilaire); rainbow trout, *Oncorhynchus mykiss* (Walbaum); Atlantic cod, *Gadus morhua* (L.); barramundi, *Lates calcarifer* (Bloch) and yellowtail kingfish, *Seriola quinqueradiata* (Temminck & Schlegel) (Azevedo et al., 1998, 2005; Rodehutscord & Pfeffer, 1999; Watanabe et al., 2000a, 2000b; Lupatsch et al., 2003; Bureau et al., 2006; Hatlen et al., 2007; Glencross, 2008; Booth et al., 2010; Helland et al., 2010).

Many authors have demonstrated the influence of salinity on growth performance of fish (Gaumet et al., 1995, Woo & Kelly, 1995; Dutil et al., 1997). Previous studies considering the effect of salinity on fish growth and metabolism have yielded controversial results. Higher growth and lower metabolic rates were recorded at isotonic salinities (Gutt, 1985; Febry & Lutz, 1987; Waller, 1992, Watanabe et al., 1993; Lambert, 1994) and this phenomenon was hypothesized to be caused by a reduction in the metabolic cost of iono- and osmoregulation. In contrast, results from Morgan &

Iwama (1991) and Sampaio et al. (2001) showed that rearing fish at an isotonic salinity did not enhance growth or reduced metabolic rates. Moreover, Tang et al. (2006) reported that there are no differences in metabolic rate of juvenile turbot at different salinities after 48 hours of acclimatisation. Turbot is considered to be a relatively euryhaline species and can adapt to salinities ranging between 10-40 g L^{-1} with a minimum level reported to be at 5-6 g L^{-1} (Waller 1992; Gaumet et al., 1995; Person Le-Ruyet, 2002; Tang et al., 2006). Furthermore, it is well known that many juveniles prefer intermediary salinities, as found, e.g. in estuaries, tidal coastal areas or coastal lagoons (Gaumet et al., 1995; Boeuf & Payan, 2001). Results from Imsland et al. (2001) also suggested enhanced growth of juvenile turbot at an intermediate salinity (20 g L^{-1}), especially at the thermal range of 18-22 °C. Although several studies measured oxygen consumption and growth performance in turbot at different environmental salinities (Waller, 1992; Gaumet et al., 1995; Tang et al., 2006) no data are available on how these changes affect the maintenance energy requirement as well as the efficiency of energy utilization for growth. In the current study the oxygen consumption (metabolic rate; including the energy expenditure for spontaneous activity) of fed fish was studied, because it is considered to be the metabolic state most closely representative of land-based aquaculture (MacIsaac, 1997). This is also termed "routine oxygen consumption" (ROC) (Jobling, 1994).

The present study intended to determine DE_m, ME_m and $k_{g\ (DE)}$, $k_{g\ (ME)}$ of juvenile turbot based on oxygen consumption and using increasing feeding levels. In addition, the effect of various salinities (10, 20, 30 g L^{-1}) on oxygen consumption as well as DE_m, ME_m and $k_{g\ (DE)}$, $k_{g\ (ME)}$ was studied.

2. Material and methods

2.1 Fish

Juvenile turbot, descendent from a domestic Norwegian broodstock, were used for the experiment and received from the hatchery Maximus A/S (Bedsted, Denmark). Fish were transferred to the rearing facilities of Gesellschaft für Marine Aquakultur, GMA (Büsum, Germany) on 28th september in 2010.

2.2 Experimental diet

A fish meal based diet was formulated to be similar to a commercial turbot feed considering gross energy (GE), macro- and micronutrients. Referring to Kaushik (1998a) and Peres & Oliva-Teles

(2008) the diet was formulated to meet the indispensable amino acid (IAA) requirements of turbot. Titanium dioxide (TiO_2) was added to the diet (10 g kg^{-1}) as an inert marker to determine the digestibility of nutrients and energy. Detailed information about the formulation and chemical composition of the experimental diet is given in Table 2-1. The diet was supplied as pellets (4 mm diameter; pellet press 14-175, AMANDUS KAHL GmbH & Co. KG, Hamburg, Germany).

Table 2-1:

Diet formulation and chemical composition of the experimental diet (g or MJ kg^{-1})

Diet formulation	
Herring fish meal LT[a]	748
Fish oil[a]	75
Wheat starch (pregelatinized)[b]	80
Wheat gluten[b]	80
Vitamin premix[c]	3.5
Mineral premix[d]	3.5
Titanium dioxide (TiO_2)[e]	10
Chemical composition	
Dry matter (DM)	933
In DM	
Crude protein (N × 6.25)	586
Crude lipid	164
Crude ash	160
Gross energy (GE)	21.8
Ca	41
P	23
TiO_2[f]	10

[a] Vereinigte Fischmehlwerke Cuxhaven GmbH & Co. KG, Cuxhaven, Germany; [fish meal made from herring offal: DM, Crude protein, crude lipid, crude ash = 948; 724, 127, 114 g kg^{-1}, respectively; LT = low-temperature cooking and drying (80-90 °C)]

[b] Kröner Stärke – H. Kröner GmbH, Ibbenbüren, Germany; (Wheat gluten: DM, Crude protein = 920, 780 g kg^{-1}, respectively)

[c] MicroMineral (supply per kg of diet): 1.4 mg $CoSO_4$; 8.75 mg $MnSO_4$; 1.75 mg CaI_2; 8.75 mg $CuSO_4$; 0.09 mg Se

[d] Vitamin Standard (supply per kg of diet): 3500 I.U. vitamin A; 700 I.U. vitamin D3; 140 mg vitamin E (alpha-Tocopherol-acetate); 14 mg vitamin K3 (Menadione); 14 mg vitamin B1; 28 mg vitamin B2; 14 mg vitamin B6; 0.03 mg vitamin B12; 210 mg vitamin C (Monophosphate); 28 mg vitamin B3; 140 mg vitamin B7; 5.60 mg Folic Acid; 0.35 mg Biotin; 140 mg Inositol; 700 mg Betain-anhydrate; 98 mg $ZnSO_4$; 140 mg Etoxyquin
(contents of vitamins, minerals and additives as specified by the manufacturer)

[e] Kronos Titan GmbH & Co.oHG, Nordenham, Germany

[f] Calculated by: $TiO_2 = Ti \times 1.67$

2.3 Respirometer system

The experiment was carried out in 10 cylindrical tanks (250 L, bottom surface: ~ 0.52 m^2) of a flow through respirometer system (Fig. 2-1) at Gesellschaft für Marine Aquakultur, GMA (Büsum, Germany). Tanks were continuously supplied with water from a recirculation system equipped with sedimentation tanks, trickling biofilter, sump (~ 1500 L) and pump, whereby each tank had a separate in- and outflow.

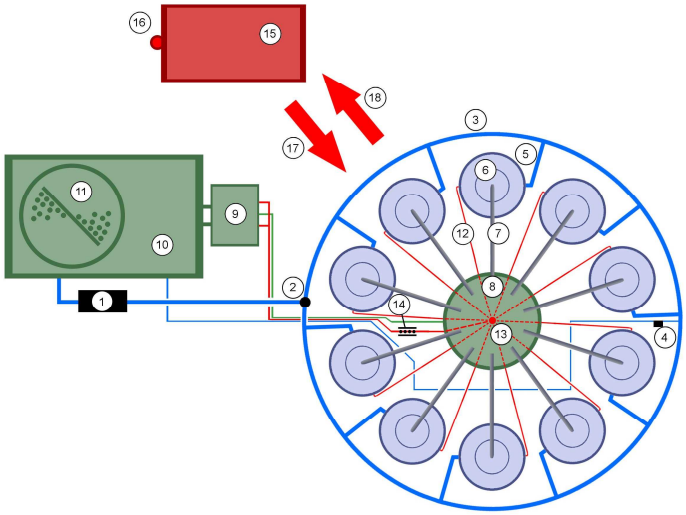

Fig. 2-1. General design of the respirometer system (© K. T. Stiller 2010)
1. Recirculation pump, 2. Manometer, 3. Water cycle, 4. Overpressure valve, 5. Fresh water inflow, 6. Respirometer (fish tank), 7. Overflow tube, 8. Sedimentation barrel, 9. Sedimentation tank, 10. Sump, 11. Trickling filter, 12. Sampling tube to the sensors, 13. Merger of sampling tubes, 14. Sensors (pH, CO_2, O_2), 15. Online control unit, 16. Main switch, 17. Online control, 18. Data transfer

Flow rate through the tanks was adjusted to be 150 l h^{-1}. For each tank in- and outflow could be individually regulated by valves controlled by a central computer system, thus, a single record of oxygen concentration, temperature and flow rate was possible for every tank. The same sensor unit was used for every measurement. Additionally, each tank equipped with an extra circulation pump (Ocean Runner OR 2500, AB Aqua Medic GmbH, Bissendorf, Germany) with separate in- and outflow to sustain a constant mixing of the water body and to cluster uneaten feed or feces to the central settling funnel at the bottom of the tanks. Uneaten feed and feces could be removed by an outlet valve. To minimize external influences inducing stress, both sides and the back of the tanks are made of opaque PVC. The front is made of transparent PVC allowing observation of the fish. Detailed information and descriptions of the respirometer system were given by Stiller (2010).

2.4 Experimental design

A restricted ration level (RRL) experiment realised at three different salinities (10, 20, 30 g L^{-1}) including three feeding levels (I-III) was designed. Feeding levels I-III corresponded to a daily feed supply of 0.3, 0.6, 0.9% of body weight (BW). Nine days prior the start of the trial 78 juvenile turbot were devided into nine balanced experimental groups (six fish each) having a similar BW. The remaining fish as well as the experimental groups were stocked in separate 50 L aquaria at a salinity of 10 g L^{-1} and fed the experimental diet at feeding level III once daily for acclimatisation to experimental conditions. Before transferred to the nine respirometer tanks, turbot of the experimental groups were individually weighed again to ensure similar BW of groups. Mean initial BW in each respirometer tank was 985 ± 10 g (164 ± 2 g BW per fish). After transferring to the respirometer fish were acclimated to the tanks at a salinity of 10 g L^{-1} for five days. Experimental groups were fed once a day per hand (11:30 - 12:15 hours) with three replications at every feeding level. To ensure that all supplied feed was consumed the central collecting funnel at the tank bottom was covered during feeding. Prior every feeding feces were eliminated from the tanks. Additionally, all uneaten pellets were removed from the respirometer tanks nearly 10 minutes after feeding and quantified. To calculate the actual feed intake the average weight of one dry pellet (determined prior the start of experiment) was multiplied with the number of uneaten pellets. Fish not used for the respirometry trial were still stocked in the aquaria at similar salinities like the experimental groups and fed at feeding level III. After acclimatisation of the experimental groups ROC was measured at the salinity level of 10 g L^{-1} for a 24 h period. Thereafter fish were starved for three days and oxygen consumption (starving oxygen consumption, SOC) was recorded (24 h). The same procedure was repeated at salinities of 20 as well as 30 g L^{-1} and represented one measuring period. Every period was followed by seven days of adaption to the next higher salinity. Therefore, feeding continued and salinity was increased stepwise within four days to left over three days for acclimatisation at the target salinity prior the next measuring period. The lower and intermediate salinities (10 and 20 g L^{-1}) were formulated by mixing tap water with seawater (North Sea, Büsum, Germany) and the higher salinity (30 g L^{-1}) by adding commercial seasalt for fish keeping („Preis sea salt“, Preis-Aquaristik, Bayerfeld, Germany) to seawater. During the experiment temperature was on average 16.5 ± 0.2 °C and dissolved oxygen ranged between 8.9 - 6.6 mg L^{-1}. Thus, the oxygen concentration never deceeded the minimum oxygen (6.0 - 7.0 mg L^{-1}) required for maximum growth of turbot (Boeuf et al., 1999; Person Le-Ruyet, 2002). The mean total ammonia nitrogen (TAN) concentration was 0.1 ± 0.1 mg L^{-1} and never exceeded 0.2 mg L^{-1}. Mean nitrite nitrogen (NO_2-N) was 0.4 ± 0.3 mg L^{-1} with a maximum of 1.0 mg L^{-1}. The photoperiod was set on a 14 h L:10 h D cycle (daybreak at 06:00 hours). The total experiment lasted 35 days.

2.5 Measurements and sampling

Fish were weighed individually at the beginning and at the end of the experiment to calculate the average BW per fish at the oxygen consumption measurements based on the specific growth rate. Fish starved at least one day prior to weighing.

To determine oxygen consumption the oxygen concentrations in the respirometer tanks as well as flow rate and temperature were automatically recorded by the computer system every hour in each experimental group for a 24 h period. Thereby the respirometer tanks were successively measured (time interval per tank: 6 min) within one hour and the time at the end of this cycle taken to be representative for each tank. One tank was left without fish and used as reference representing the inflow oxygen concentration by the system for all experimental tanks within the hourly record cycle as well as to correct for oxygen consumption by microorganism inhabiting the system. To avoid any effect of fouling processes on oxygen concentration the reference tank was carefully cleaned before the start of each 24 h measuring period. Prior to every 24 h measurement the sensor (JUMO dTRANS O2 01, JUMO GmbH & Co. KG, Fulda, Germany) was calibrated on humidified air (saturation 100%; immediately above the water surface). To ensure an adequate water quality as well as to verify the recording of the computer system temperature (OxyGuard-Handy Polaris 2, OxyGuard International A/S, Birkerød, Denmark), salinity (HI 96822 digital seawater refractometer, HANNA instruments Inc., Woonsocket-RI-USA), TAN and NO_2-N (colorimetric test kit - Microquant; Merck KgaA, Darmstadt, Germany) were manually measured every day.

Following the respirometer trial the experimental fish and the remaining turbot from aquaria were used for determination of nutrient and energy digestibility of the diet. Fish were devided in three subsets. Subsets consisted of two replicates and were acclimated to salinities of 10 (n = 12 fish per replicate), 20 (n = 12 fish per replicate) and 30 g L^{-1} (n = 15 fish per replicate), respectively. At each salinity level fish were fed 12 days once daily with 1.0% of BW. Approximately 12 h after the last feeding the content of the posterior intestine (from the ileocaecal valve to the anus) was carefully sampled by dissection once fish were killed by an overdose of MS-222 (200 mg L^{-1}). No abrasion of intestinal walls was performed to minimize the contamination of digesta with epithelial cells. Due to limited quantities to do accurate chemical analyses the sampled intestinal contents were pooled for each salinity level, stored in a plastic container at -18 °C and subsequently freeze-dried. Prior to chemical analyses intestinal samples were homogenized.

2.6 Chemical analyses

According to Naumann & Bassler (1976) the experimental diet and intestinal contents (due to the small quantity except for crude lipid) were analyzed in duplicate for dry matter (DM), crude ash (CA), crude lipid (CL) and gross energy (GE; adiabatic bomb calorimeter C200, IKA-Werke GmbH & Co.KG, Staufen, Germany). CL analysis was done with HCl hydrolysis. Crude protein (CP, N × 6.25) was determined using the Dumas combustion principle (True Spec® N, LECO Corporation, St. Joseph, MI, USA). TiO_2 in the diet and intestinal contents was determined according to Brandt & Allam (1987): TiO_2 was solubilized by the Kjeldahl procedure for 3 h in 0.96 g g^{-1} sulphuric acid, then 0.35 g g^{-1} hydrogen peroxide (H_2O_2) was added to the filtered TiO_2 solution to form a yellow complex and color intensity measured in a spectrophotometer (visible spectrophotometer 6300, Barloworld Scientific Ltd. T/As Jenway, Dunmow, Essex, U.K.) at 405 nm. The determination of calcium and phosphorus contents of the diet was performed according to VDLUFA VII 2.2.2.6 method (VDLUFA, 2008).

2.7 Definitions, calculations and statistics

Specific growth rate (SGR), % per day: $100 \times (\ln BW_1 - \ln BW_0) \times days_{exp}^{-1}$, where $days_{exp}$ are the experimental days and BW_0 and BW_1 are initial and final mean BW, respectively.

Metabolic body weight (MBW), $kg^{0.8}$: $BW_t^{0.8}$, where BW_t represent the average BW (kg) of fish at each 24 h measuring period. BW_t was calculated based on SGR. According to our previous statements (Dietz et al., 2012) the common metabolic weight exponent for energy of 0.8 was applied.

Apparent digestibility coefficients (ADC), %: $100 \times [1 - (\%\ feed_{TiO2} / \%\ feces_{TiO2}) \times (\%\ feces_n / \%\ feed_n)]$, where subscript n stands for a specific nutrient or energy.

To calculate energy intake from digestible protein (DEI_p) the energetic equivalent of 23.6 kJ g^{-1} protein (Blaxter, 1989) was used.

Non-fecal N excretion was calculated using the ratio between retained protein (RP) and digestible protein intake (DPI) from a previous experiment using turbot descendent from the same broodstock and a similar diet (Dietz et al., 2012). The non-fecal N excretion was derived from the difference between digestible N intake (DPI/6.25) and N deposition (RP/6.25). The energy loss associated with this excretion was calculated using the energetic equivalents of 24.9 kJ g^{-1} ammonia N and 23.1 kJ g^{-1} urea N (Elliott & Davison, 1975). Assuming 15% of N is excreted as urea and 85% as ammonia (Dosdat et al., 1995) results in 24.6 kJ g^{-1} non-fecal N.

Oxygen consumption (OC, mg h^{-1}) was calculated according to the following equation: $OC = (cO_2^r - cO_2^i) \times F$, where cO_2^r and cO_2^i are the concentrations of dissolved oxygen (mg L^{-1}) in the reference tank and individual repirometer tanks, respectively. F defines the flow rate (L h^{-1}) through the individual respirometer tanks. ROC and SOC reflecting the average OC per MBW for fed and starving fish within 24 h, respectively. To consider the unsteady-state situation in the flow-through system the calculated OC-values were verified by applying the following equation proposed by Niimi (1978):

$$Q_{O2} = [((F \times c_0 \times e^{-F/V \times t}) - (F \times c))/(1 - e^{-F/V \times t})] + (F \times c_{in}) \qquad (1)$$

where, referring to the present study, Q_{O2} represent OC, c_0 and c are cO_2^i of two consecutive records, F = 150 L h^{-1}, V = 250 L, t is the time intervall between the records of c_0 and c (= 1 h) and c_{in} the mean between cO_2^r related to records of c_0 and c, respectively.

Specific dynamic action (SDA, mg $kg^{-0.8}$ h^{-1}): SDA = ROC-SOC. SDA represents the OC above starving and reflects the energy requirement accociated with feeding, e.g. searching, ingestion, digestion and absorbtion of feed.

Heat (energy used for SDA and maintenance inclusive spontaneous activity) was calculated based on OC (13.6 kJ per g O_2 consumed; Elliott & Davison, 1975).

All data considering energy metabolism were presented per MBW.

Digestible energy intake (DEI), metabolizable energy intake (MEI) and RE (RE = MEI - Heat) at feeding levels I-III were subjected to linear regression analysis for determination of DE_m, ME_m, $k_{g\ (DE)}$ and $k_{g\ (ME)}$ according to the following linear regression equations:

$$RE\ (kJ\ kg^{-0.8}\ d^{-1}) = a + k_{g\ (DE)} \times DEI\ (kJ\ kg^{-0.8}\ d^{-1})$$

$$RE\ (kJ\ kg^{-0.8}\ d^{-1}) = a + k_{g\ (ME)} \times MEI\ (kJ\ kg^{-0.8}\ d^{-1})$$

Energy intakes at RE = 0 are defined as the maintenance requirements, and the efficiencies of energy utilization for growth above maintenance are given by the slopes. However, this statistical approach does not provide the standard errors for the estimates of the energy maintenance requirements, which are necessary for testing the significance of differences. Therefore, DE_m and ME_m were alternatively determined by the following equations, where energy intake is taken as dependent and RE as independent variable:

$DEI\ (kJ\ kg^{-0.8}\ d^{-1}) = DE_m + 1/k_{g\ (DE)} \times RE\ (kJ\ kg^{-0.8}\ d^{-1})$

$MEI\ (kJ\ kg^{-0.8}\ d^{-1}) = ME_m + 1/k_{g\ (ME)} \times RE\ (kJ\ kg^{-0.8}\ d^{-1})$

Here, DE_m and ME_m are represented by the y-intercepts and the slopes $1/k_{g\ (DE)}$ and $1/k_{g\ (ME)}$ - the reciprocals of the k_g -values - describe the costs for DE or ME (kJ) per unit of RE (kJ), respectively. Because DE_m, ME_m, $k_{g\ (DE)}$ and $k_{g\ (ME)}$ based on ROC it must be noted that DE_m and ME_m -values determined by extrapolation at RE = 0 include also the energetic requirements for spontaneous activity.

All the data were checked for homogeneity of variance using box plots and residual plots. DEI, MEI and RE were subjected to analysis of covariance (ANCOVA) and multiple comparisons were used to compare slopes (Holm) and y-intercepts (Tukey) of the linear regression equations, respectively. Thereby, the influence of tank as random factor could not be included in the statistical model. Results of growth and energy budget parameters were analyzed by ANOVA applying linear mixed models (y ~ salinity + feeding level + salinity × feeding level + tank as random factor) regarding heterogenous variances and followed by all-pair comparisons between salinities within feeding levels as well as between feeding levels within salinities. Differences were considered to be significant at the $p < 0.05$ level. All statistical data analyses were performed using R-Software version 2.11.1 (R Development Core Team, 2010). Results are presented as mean ± SD.

3. Results

No fish died during the experiment.

3.1 Digestibility

The estimated apparent digestibilities of nutrients and energy of the diet are shown in Table 2-2. Despite the lack of error terms, the digestibility of DM, organic matter (OM), CP, CA and energy appeared to decrease with increasing salinity.

Table 2-2:

ADC[a] (%) of nutrients and energy in juvenile turbot at different salinities

Salinity [g L^{-1}]	10	20	30
Dry matter	73.4	69.8	68.9
Organic matter[b]	83.7	80.5	79.8
Crude protein	86.6	84.2	83.8
Crude ash	19.4	13.5	11.6
Energy	87.1	84.3	83.9

[a] ADC, apparent digestibility coefficients: calculated by using one pooled sample of intestinal content of fish fed at salinities of 10 (n = 24), 20 (n = 24) and 30 (n = 30) g L^{-1}

[b] Calculated in diet and intestinal contents by: Organic matter = dry matter – crude ash

3.2 Feed intake and oxygen consumption

Feed intake as well as the results of OC measurements are presented in Table 2-3. No differences were observed in feed intake between the different salinities.

The deviations between results of ROC and SOC determined by using the actual data of OC and their estimates based on the unsteady-state mass balance (equation (1)) are less than 2.5%. ROC and SDA increased with increasing feeding level ($p < 0.001$), whereas SOC was independent from feeding level. No differences could be observed in ROC and SDA between salinities except at feeding level III, where ROC was reduced at higher salinities (20, 30 g L^{-1}) and SDA showed the highest reduction at a salinity of 20 g L^{-1}. SOC of fish previous fed at feeding levels II and III was higher at a salinity level of 20 g L^{-1} than at 10 or 30 g L^{-1}. SOC was consistently lower than ROC at each salinity and feeding level ($p < 0.05$).

The diurnal course of oxygen consumption showed similar patterns between salinities (Fig. 2-2). There was an increase in SOC from 07:00-12:00 hours, whereas the increase in ROC was extended from 07:00-15:00 hours. SOC and ROC continously decreased in the diurnal course and the lowest values obtained in the early morning (05:00-07:00 hours). SOC and ROC converged immediately before feeding (11:30-12:15 hours).

Table 2-3:

Body weight, feed intake and oxygen consumption of fed and starved juvenile turbot at three different salinities

Salinity [g L^{-1}]	FL	Body weight[a] [g]	Feed intake[b] [g DM $kg^{-0.8}d^{-1}$]	ROC	SOC [mg $kg^{-0.8}h^{-1}$]	SDA
10	I	165 ± 1	2.08 ± 0.05^{A}	61.0 ± 14.3^{A}	43.3 ± 10.3	17.7 ± 5.1^{A}
	II	168 ± 3	4.19 ± 0.01^{B}	70.6 ± 8.5^{AB}	38.6 ± 6.8^{x}	32.0 ± 2.8^{A}
	III	174 ± 3	6.19 ± 0.18^{C}	84.9 ± 4.2^{Bx}	38.7 ± 3.0^{x}	46.2 ± 4.0^{Bx}
20	I	168 ± 1	2.12 ± 0.01^{A}	56.4 ± 9.7	40.6 ± 6.4	15.8 ± 10.0
	II	176 ± 3	4.21 ± 0.01^{B}	70.0 ± 10.5	43.6 ± 4.6^{y}	26.4 ± 6.0
	III	187 ± 3	5.99 ± 0.27^{C}	76.1 ± 6.0^{y}	42.6 ± 1.6^{y}	33.4 ± 4.5^{y}
30	I	170 ± 1	2.10 ± 0.05^{A}	47.8 ± 4.0^{A}	30.5 ± 7.6	17.3 ± 5.3^{A}
	II	184 ± 4	4.19 ± 0.02^{B}	68.2 ± 4.2^{AB}	36.2 ± 5.6^{x}	32.0 ± 3.2^{AB}
	III	202 ± 4	5.66 ± 0.73^{C}	74.7 ± 2.0^{By}	35.2 ± 2.9^{x}	39.5 ± 2.8^{Bxy}

Values are means ± SD of triplicate feeding groups; within each column values with different superscript letters are significantly different ($p < 0.05$); capital letters showing contrasts in oxygen consumption between feeding levels within salinity; small letters showing contrasts in oxygen consumption between salinities within feeding level; ROC, routine oxygen consumption (fed fish); SOC, starving oxygen consumption; SDA, specific dynamic action (= ROC - SOC)

[a] Calculated from estimated weights on measuring days of ROC and SOC (n = 6)

[b] On measuring days of ROC

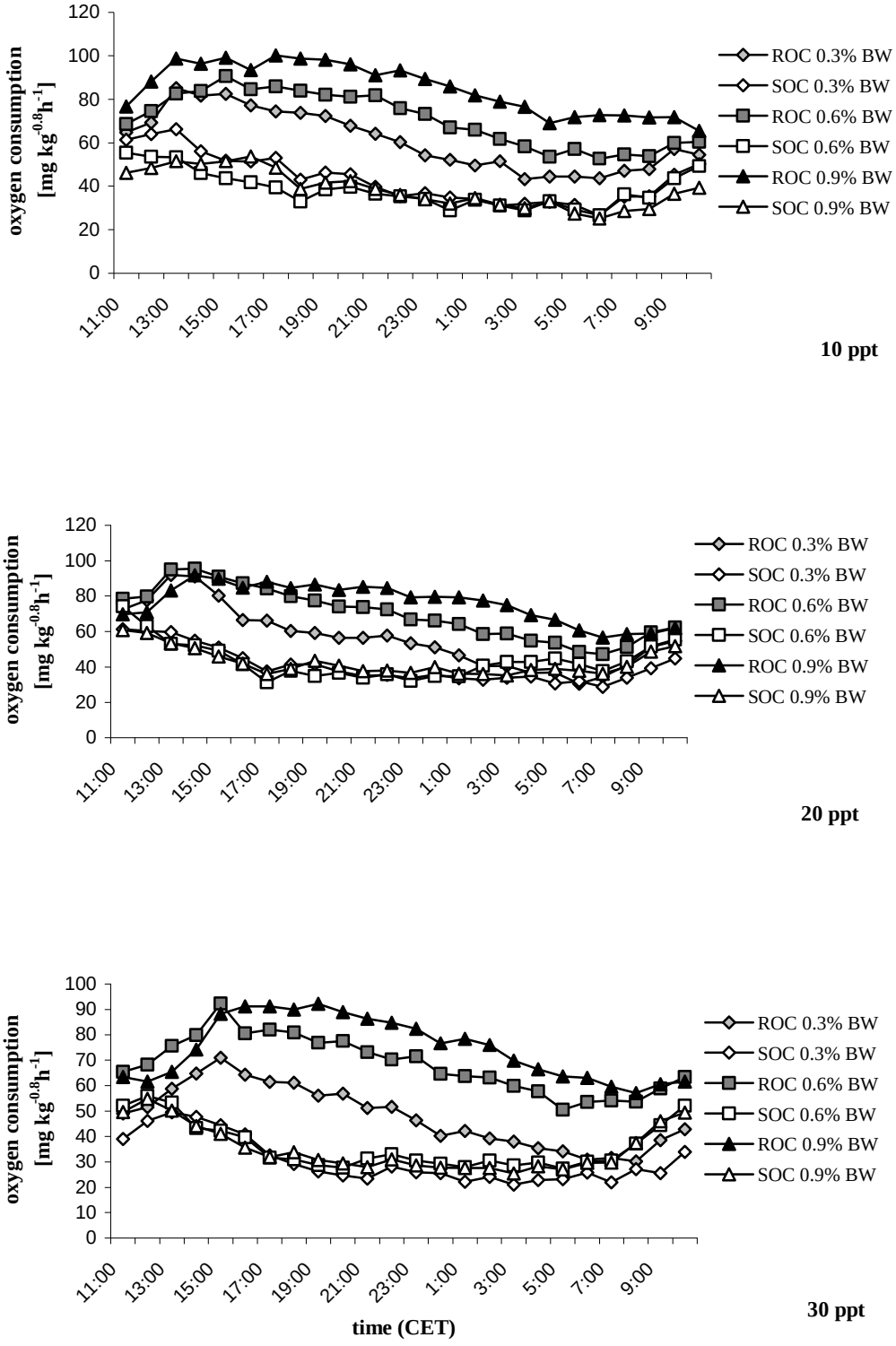

Fig. 2-2. Diurnal course of oxygen consumption of juvenile turbot fed at three different feeding levels (0.3, 0.6, 0.9% BW) at salinities of 10, 20 and 30 g L^{-1}. Each data point represents the mean of triplicates. ROC, routine oxygen consumption (fed fish); SOC, starved oxygen consumption; BW, body weight; CET, Central European Time

3.3 Energy budget

The energy budgets of the experimental groups are shown in Table 2-4. GEI at feeding levels I-III were similar between salinities. 12.9%, 15.7% and 16.1% of GEI were lost as fecal energy at salinities of 10, 20 and 30 g L^{-1}, respectively. Energy excretion via ammonia and urea significantly increased as DEI_p increased with feeding level. In average, energy loss by ammonia and urea excretion amounted 6.4% of DEI (5.5% of GEI), resulting in MEI-values representing approximately 93.6% of DEI. DEI, MEI, DEI_p and E_N-loss tended to decrease with increasing salinity, however, differences were only determined to be significant at feeding level II due to lower variations. RE was similar between salinities at each feeding level and increased with increasing feeding level.

At feeding level III 74.7 - 76.1% of MEI was converted into body energy, whereas only 45.3-56.1% was used in level I due to a higher proportion of ME_m.

Table 2-4:

Energy budget of juvenile turbot at three different salinities

Salinity [g L^{-1}]	FL	GEI	DEI	MEI	DEI_p	E_N-loss	RE	ME_m/MEI	RE/MEI
		[kJ $kg^{-0.8}d^{-1}$]						[%]	
10	I	45.2 ± 1.1^{A}	39.4 ± 1.0^{A}	36.5 ± 0.9^{A}	24.9 ± 0.6^{A}	2.9 ± 0.1^{A}	16.6 ± 5.3^{A}	50.2 ± 1.3^{Ax}	45.3 ± 13.7^{A}
	II	91.3 ± 0.2^{B}	79.5 ± 0.2^{Bx}	74.8 ± 0.2^{Bx}	50.2 ± 0.1^{Bx}	4.8 ± 0.0^{Bx}	51.7 ± 2.8^{B}	24.5 ± 0.1^{Bx}	69.2 ± 3.7^{B}
	III	134.9 ± 4.0^{C}	117.5 ± 3.5^{C}	110.6 ± 3.3^{C}	74.1 ± 2.2^{C}	6.9 ± 0.2^{C}	82.9 ± 4.6^{C}	16.6 ± 0.5^{C}	74.9 ± 2.0^{B}
20	I	45.2 ± 0.1^{A}	38.9 ± 0.1^{A}	36.0 ± 0.1^{A}	24.6 ± 0.1^{A}	2.9 ± 0.0^{A}	17.6 ± 0.1^{A}	48.0 ± 0.1^{Ax}	48.9 ± 8.8^{A}
	II	91.6 ± 0.1^{B}	77.3 ± 0.1^{By}	72.6 ± 0.1^{By}	49.0 ± 0.1^{By}	4.7 ± 0.0^{By}	49.8 ± 3.4^{B}	23.8 ± 0.0^{By}	68.5 ± 4.7^{B}
	III	130.5 ± 5.9^{C}	110.1 ± 5.0^{C}	103.6 ± 4.7^{C}	69.8 ± 3.2^{C}	6.5 ± 0.3^{C}	78.8 ± 3.4^{C}	16.7 ± 0.8^{C}	76.1 ± 1.2^{B}
30	I	45.7 ± 1.2^{A}	38.3 ± 1.0^{A}	35.5 ± 0.9^{A}	24.3 ± 0.6^{A}	2.8 ± 0.1^{A}	19.9 ± 0.5^{A}	37.5 ± 1.0^{Ay}	56.1 ± 2.7
	II	91.2 ± 0.4^{B}	76.5 ± 0.3^{Bz}	71.9 ± 0.3^{Bz}	48.5 ± 0.2^{Bz}	4.6 ± 0.0^{Bz}	49.6 ± 1.1^{B}	18.5 ± 0.1^{Bz}	69.0 ± 1.8
	III	123.3 ± 16.0^{C}	103.5 ± 13.4^{C}	97.4 ± 12.6^{C}	65.5 ± 8.5^{C}	6.1 ± 0.8^{C}	73.0 ± 12.4^{C}	13.8 ± 1.9^{C}	74.7 ± 3.4

Values are means ± SD of triplicate feeding groups; within each column values with different superscript letters are significantly different ($p < 0.05$); capital letters showing contrasts between feeding levels within salinity; small letters showing contrasts between salinities within feeding level; FL, feeding level; GEI, gross energy intake, DEI, digestible energy intake; MEI, metabolizable energy intake; DEI_p, energy intake from digestible protein; E_N, energy of non-fecal N (ammonia + urea); RE, retained energy (protein and fat); ME_m, maintenance energy requirement of ME estimated in the present study (18.3, 17.3 and 13.3 kJ $kg^{-0.8}d^{-1}$ at salinities of 10, 20 and 30 g L^{-1}, respectively)

3.4 Maintenance energy requirement and efficiency of energy utilization for growth

The linear relationships between RE (kJ $kg^{-0.8}d^{-1}$) and DEI (kJ $kg^{-0.8}d^{-1}$) as well as RE and MEI (kJ $kg^{-0.8}d^{-1}$) are shown by Fig. 2-3 and Fig. 2-4, respectively, and described by the following linear regression equations (means ± SE):

10 ppt: $RE = -16.7 (\pm 2.2) + 0.851 (\pm 0.026) \times DEI$ (2)
($R^2 = 0.99$)

20 ppt: $RE = -15.9 (\pm 2.3) + 0.857 (\pm 0.029) \times DEI$ (3)
($R^2 = 0.99$)

30 ppt: $RE = -12.0 (\pm 2.4) + 0.819 (\pm 0.031) \times DEI$ (4)
($R^2 = 0.99$)

10 ppt: $RE = -15.9 (\pm 2.2) + 0.897 (\pm 0.028) \times MEI$ (5)
($R^2 = 0.99$)

20 ppt: $RE = -15.2 (\pm 2.3) + 0.903 (\pm 0.030) \times MEI$ (6)
($R^2 = 0.99$)

30 ppt: $RE = -11.3 (\pm 2.4) + 0.862 (\pm 0.032) \times MEI$ (7)
($R^2 = 0.99$)

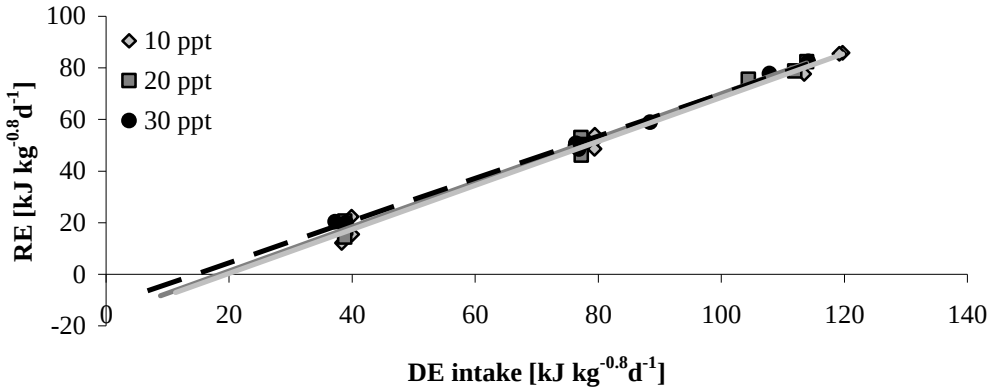

Fig. 2-3. Daily energy retention per metabolic body weight (RE) of juvenile turbot fed increasing levels of digestible energy (DE) at three different salinities [10, 20, 30 g L^{-1}]. Each data point represents the mean of one respirometer tank; equations (2)-(4) are given in the text.

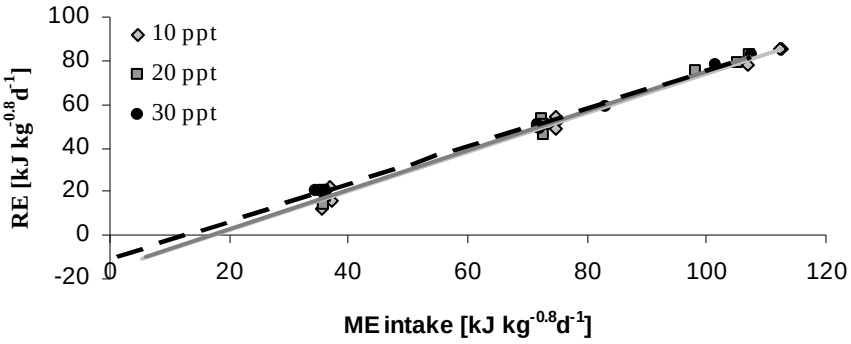

Fig. 2-4. Daily energy retention per metabolic body weight (RE) of juvenile turbot fed increasing levels of metabolizable energy (ME) at three different salinities [10, 20, 30 g L^{-1}]. Each data point represents the mean of one respirometer tank; equations (5)-(7) are given in the text.

Results of linear regression of the relationship between DEI (y) and RE (x) as well as MEI (y) and RE are summarized in Table 2-5. The determined $k_{g\ (DE)}$-values of 0.860 ($1/k_{g\ (DE)}$ = 1.16), 0.865 ($1/k_{g\ (DE)}$ =1.16) and 0.821 ($1/k_{g\ (DE)}$ = 1.22) at salinities of 10, 20 and 30 g L^{-1} were similar to corresponding $k_{g\ (DE)}$-values of linear regression equations (2)-(4). Thus, results of statistical comparisons between $1/k_g$-values and between k_g-values were similar, too. $K_{g\ (DE)}$ and DE_m did not differ significantly between salinities, but it seems that these values decreased at a salinity of 30 g L^{-1}. Similar statistical correlations were obtained when considering the relationship between MEI and RE.

Table 2-5:

Parameters of the linear regression equations (n=9)[a] between either DEI and RE: DEI (kJ $kg^{-0.8}$ d^{-1}) = DE_m + $1/k_{g\ (DE)}$ × RE (kJ $kg^{-0.8}$ d^{-1}) or MEI and RE: MEI (kJ $kg^{-0.8}$ d^{-1}) = ME_m + $1/k_{g\ (ME)}$ × RE (kJ $kg^{-0.8}$ d^{-1})

Salinity [g L^{-1}]	DE_m [kJ $kg^{-0.8}d^{-1}$]	$1/k_{g\ (DE)}$	ME_m [kJ $kg^{-0.8}d^{-1}$]	$1/k_{g\ (ME)}$
10	20.2 (± 2.2)	1.16 (± 0.04)	18.3 (± 2.0)	1.10 (± 0.03)
20	19.1 (± 2.2)	1.16 (± 0.04)	17.3 (± 2.0)	1.10 (± 0.04)
30	14.9 (± 2.3)	1.22 (± 0.04)	13.3 (± 2.1)	1.16 (± 0.04)

[a] R^2 = 0.99, $p < 0.001$, similar for all salinities

Values are mean ± SE; DEI, digestible energy intake; MEI, metabolizable energy intake; RE, retained energy; DE_m, maintenance requirement of digestible energy, ME_m, maintenance requirement of metabolizable energy; $1/k_{g\ (DE)}$, energetic costs of DE (kJ) per unit RE; $1/k_{g\ (ME)}$, energetic costs of ME (kJ) per unit RE (kJ)

4. Discussion

4.1 Dietary digestibility

ADC-values in the current study based on the determinations at one feeding level only (1% BW). Commonly, most studies show that feeding levels have no impact on digestibility of nutrients (assuming that feed intake is not below levels needed to support reasonable growth rates) and intestinal absorptive capacity is not likely to be a limiting factor for nutrient digestibility (NRC, 2011). Therefore, the use of the same ADC-values of nutrients and energy for all feeding levels in the present study is considered to be acceptable. Due to the low cohesion accompanied with dissolution in the water body it was very difficult to collect feces from turbot. Similar difficulties in the collection of feces (digesta) were reported in other flatfish (Grisdale-Helland & Helland, 1998; Dias et al., 2010). Referring to a previous study (Dietz et al., 2012) it was decided to use the method of dissection the hind gut (12 h after the last meal) combined with a marker to obtain accurate samples of digesta. Due to low amounts of digesta sampled in the hind gut, it was impossible to realize chemical analyses within each replicate. Thus, we decided to pool the samples from all replicates within each salinity. Therefore, statistical analysis was not possible and consequently resulted in estimated ADC-values. Although using no replicates the observed ADC-values can be considered as representative as ADC-values of dietary nutrients and energy were in the range of other studies with turbot (Oliva-Teles et al., 1999; Regost et al., 2001, 2003; Peres & Oliva-Teles, 2005; Dietz et al., 2012) and Atlantic halibut, *Hippoglossus hippoglossus* (L.) (Berge & Storebakken, 1991; Grisdale-Helland & Helland, 1998) at similar dietary composition. Small variations of ADC-values between studies may be caused by using different feces collecting methods. Nevertheless, our observations of an apparent reduction of nutrient and energy digestibilities with increasing salinity correspond with results from studies in other fish species (MacLeod, 1977; Ferraris et al., 1986; Ringø, 1991; Woo & Kelly, 1995; Storebakken et al., 1998).

Teleost fish in seawater are known to compensate osmotic water losses at increasing salinities by intensified drinking rates (Boeuf & Payan, 2001), which could consequently increase gastric emptying rate, intestinal passage rate and pH in the digestive tract as well as decrease feed breakdown and absorbtion of nutrients and energy (MacLeod, 1977; Ferraris et al., 1986; Usher et al., 1990). Furthermore, Boeuf & Payan (2001) presumed that digestive enzymes are affected by water content in the gut and Woo & Kelly (1995) as well as Moutou et al. (2004) reported a higher trypsin activity at lower salinities in *Sparus sarba* (Forsskål) and *Sparus aurata*, respectively. Although not directly comparable to the fish in presented experiment Brown & Tytler (1993) observed higher drinking rates and implied changes in skin and gill permeability in turbot larvae at a higher salinity. Therefore, it is possible that the salinity related decreases in digestibility of

nutrients and energy in the present study may, in part, due to higher drinking rates. In contrast, De Silva & Perrera (1984) and Dutil et al. (1997) showed that salinity had no significant effect on nutrient and energy digestibility.

The intestine is an osmoregulatory organ in seawater fish (reviewd by Kirsch et al., 1985). Marine teleostean fish selectively absorb water and minerals across the intestinal epithelium (Walsh et al., 1991). Thereby, monovalent ions (e.g. Na^+, Cl^-) are largely absorbed and divalent cations (Ca^{2+} and Mg^{2+}) are left behind (Walsh et al., 1991; Taylor & Grosell, 2006). Furthermore, intestinal fluids of most marine teleosts are alkaline (pH 8.4-9.0) and contain high levels of bicarbonate equivalents, which increases with drinking rates at elevated ambient salinities (Wilson et al., 2002). The latter authors stated that one consequence of the luminal alkalinity and high bicarbonate concentrations is precipitation of calcium and magnesium as carbonate complexes playing a potential role in water absorption and osmoregulation. Wilson et al. (2002) showed that an elevated bicarbonate secretion resulted in increased intestinal calcium precipitation in flounder, *Platichthys flesus* (L.). The previous aspects suggest that intestinal mineralization is a general feature of osmoregulation in marine teleosts (Walsh et al., 1991; Wilson et al., 2002). Accumulation of exogenous, non-dietary minerals in the lower intestine may explain the reduced digestibility of dietary ash. In this context dietary DM digestibility is actually lower to a small extent than estimated. This is because the the proportion of non-dietary ash could not be determined and consequently the error in the percentage of DM of the feces caused by non-dietary ash was not corrected for in the digestibility equation. The presence of non-dietary ash in the faeces does not affect the digestibility calculations of organic matter, CP and energy.

4.2 Feed intake and oxygen consumption

Feed intake directly affected oxygen consumption (Jobling, 1994) and consequently ROC. No entire effect of salinity on feed intake could be seen in the present experiment. Thus, ROC between salinities within feeding level was unlikely affected by differences in feed intake. According to the statements of Eriksen (2002) and due to only low deviations regarding the validation of the actual values by estimates based on unsteady-state mass balance ROC and SOC determined in the present study can be considered as representative. Furthermore, OC-values in the present experiment were similar to values determined in other studies with turbot and several flatfish species (Table 6), e.g. common dab, *Limanda limanda* (L.); Japanese flounder, *Paralichthys olivaceus* (Temminck & Schlegel); American plaice, *Hippoglossoides platessoides* (Fabricius), winter flounder,

Pleuronectes americanus (Walbaum); yellowtail flounder, *Pleuronectes ferrugineus* (Storer); California halibut, *Paralichthys californicus* (Ayres) and Starry flounder, *Paralichthys stellatus*.

Table 2-6:

Oxygen consumption of different flatfish species

Species	Fish size (g)	Feeding	Temperature (°C)	OC[a] (mg $kg^{-0.8}h^{-1}$)	Reference
Turbot	165-207	fed (0.3-0.9% BW)	16.5	61-85 (10 g L^{-1}) 56-76 (20 g L^{-1}) 48-75 (30 g L^{-1})	Present study[b]
		starved		39-43 (10 g L^{-1}) 41-44 (20 g L^{-1}) 31-36 (30 g L^{-1})	
Turbot	148	fed to satiation	14	99	Brown et al. (1984)
	229		16	111	
Turbot	350	fed to satiation	16	18-27 (10 g L^{-1}) 21-31 (19 g L^{-1}) 27-37 (27 g L^{-1}) 26-36 (35 g L^{-1})	Gaumet et al. (1995)
Turbot	100	starved	16-19	28 (8 g L^{-1}) 37 (22 g L^{-1}) 36 (29 g L^{-1})	Waller (1992)
Atl. halibut	233-454	fed	10	40-64	Davenport et al. (1990)
Flounder	395	starved	15	178	Duthie (1982)
Common dab	396	starved		142	
Japanese flounder	438	starved	20	35	Honda (1988)
American plaice	287	starved	14	23	MacIsaac et al. (1997)
Winter flounder	201			86	
Yellowtail flounder	385			75	
California halibut	165	fed (0.7% BW) farm conditions	21.6	125	Merino et al. (2009)
Starry flounder	603-764	starved	7.5-10.5	34	Wood et al. (1979)

[a] OC, oxygen consumption (details on salinity levels are given in brackets)

[b] Ranges represent the OC of the different feeding levels

Fish in an isotonic medium have the lowest standard metabolic rates, whereas osmoregulation in seawater appears to be energetically more expensive than in freshwater (Boeuf & Payan, 2001). But there are contrary reports in the literature regarding the effect of an isotonic salinity on OC (Morgan

& Iwama, 1991). The isotonic salinity for turbot is considered to be arround 10 g L^{-1} and higher ROC and SOC were observed with increasing salinity (Waller, 1986, 1992; Gaumet et al., 1995). In contrast, Tang et al. (2006) determined that ROC was independent from salinity after 48 h of adaption. The results of Tang et al. (2006) also confirm that the time allowed fish for adaption to the different salinities was adequate in the present study. Turbot in our experiment showed only less activity during the measurements of SOC and therefore this data can be considered to be close to the standard metabolic rate which reflects the basal metabolism of starving fish at zero activity (Jobling, 1994). However, for a precise determination of the standard metabolic rate in fish, a simultaneous recording of activity and OC is required (Jobling, 1994).

The reduced SOC at a higher salinity (30 g L^{-1}) was unexpected, as the energetic costs of ion transport should continue to increase as the difference between internal body concentration and ambient water concentration increases (Imsland et al., 2003). Nordlie et al. (2001) reported that the routine metabolic rates in euryhaline *Cyprinodon variegatus* (Lacépède) were highest at ambient salinities from 15-50 g L^{-1}, somewhat lower at salinities less than 15 g $L^{-1}$and markedly declined above 50 g L^{-1}. A similar pattern was observed for SOC in the present study, however, SOC seemed to decline earlier (30 g L^{-1}) and less marked. Claireaux & Lagardère (1999) also reported a reduced metabolic rate at a salinity of 30 g L^{-1} for European seabass. It must be noted that routine metabolic rates determined by Nordlie et al. (2001) and Claireaux & Lagardère (1999) were measured in starved fish at an activity level reduced to spontaneous movements and therefore are comparable to results of SOC in our study. Nordlie et al. (2001) discussed the decline in metabolic rate at higher salinieties was caused by a reduction in the osmotic permeability of the gills which resulted in a reduced potential for OC as a consequence. Boeuf & Payan (2001) also mentioned that many fish species developed impermeabilization mechanisms to avoid too great water losses through the gills by diffussion. We can only speculate at this time that the reduced SOC of turbot at a salinity level of 30 g L^{-1} had a similar reason. However, results of Waller (1992), who observed an increasing SOC between 8-35 g L^{-1} salinity in turbot, will not agree with our findings at 30 g L^{-1}. On the other hand, the reduced SOC at 10 g L^{-1} in our experiment correspond to the results of the latter author and of Gaumet et al. (1995) and supports the concept of lower energetic demands for osmoregulation at an isotonic salinity. In contrast to SOC, no clear effect of salinity on ROC was observed in the current study. This was in agreement to Tang et al. (2006), whose results in ROC indicate that energetic demands for osmoregulation in turbot are neglectable after acclimatisation. Furthermore, SDA seemed to be lower at a salinity of 20 g L^{-1} in the present study and compensated the higher SOC. Thus, the influence of salinity observed in SOC maybe covered by the effect of feeding when considering ROC.

Despite the fact that Waller (1992) showed no effect of salinity on spontaneous activity of turbot, an influence of varying activity levels (social interactions, feed searching, energy sparing) on SOC and ROC can not generally be excluded in the present study. In this context it has to be noted that, regarding to the suggestion of Nordlie et al. (2001), a lower potential for OC at high salinity due to reduced gill permeability can also reduce activity. Considering our data and the conflicting results in the literature the observed differences in SOC as well as ROC between various salinities cannot exclusively attributed to energy required for osmoregulation and may apart from spontaneous activity influenced by other undetermined physiological processes. Several studies (Febry & Lutz, 1987; Mancera et al., 1995; Boeuf et al., 1999; Boeuf & Payan, 2001; McCormick, 2001; Morgan & Iwama, 2001) mentioned the role of hormonal changes (e.g. cortisol, growth hormone, insulin-like growth factor-I, thyroid hormones), also known to affect metabolic rate and therefore OC of fish, related to changing salinities.

The diurnal courses of oxygen consumption were similar to results in a former study in turbot (Waller, 1992), except there was no second daily increase in oxygen consumtion in the evening in the present work. Our data correspond well with observations in other fish species, e.g. gilthead seabream (Guinea & Fernandez, 1997) and rainbow trout (Stiller, 2010). OC generally increases several hours after cessation of feeding (Bureau et al., 2002; Jobling, 1994) and therefore the extended increase of ROC can be attributed to SDA.

4.3 Energy budget

Energy loss by undigested feed in the present study (12.9-16.1%) was lower than results in a previous experiment (21.4%) dealing with juvenile turbot of the same strain fed a similar diet (Dietz et al., 2012). This difference maybe caused by using slightly larger fish, which seem to digest nutrients and energy more efficiently than smaller fish (Ferraris et al., 1986; Jobling, 1994). As commonly known from other studies in various fish species (Beamish & Thomas, 1984; Ballestrazzi et al., 1994; Kaushik & Medale, 1994; Dosdat et al., 1995; Vergara et al., 1996) N-excretion is directly correlated with DPI and in consequence with DEI_p. Therefore, the higher energy losses by N-excretion with increasing salinity at feeding level II can be attributed to the higher DEI_p. It must be considered that we decided to calculate E_N-loss in the present study from the RP/DPI ratio in our previous experiment (salinity: 26 g L^{-1}; Dietz et al., 2012), where similar fish and diet were used. Tang et al. (2006) observed no significant differences in ammonia excretion rates in juvenile turbot between various salinities after 48 h of adaption and therefore enabled to use

the same values of RP/DPI for salinities of 10, 20 and 30 g L^{-1} in the present study. Because not directly measured, E_N-loss and consequently MEI in our study must be considered as estimates.

The similar GEI indicates that the observed differences between salinities considering DEI and DEI_p as well as the resulting E_N-loss and MEI seemed to be basically attributed to the differences in dietary digestibility rather than induced due to osmoregulation processes. This was supported by the fact that the latter parameters decreased with increasing salinity like the nutrient and energy digestibilities. Fonds et al. (1992) suggested that 53-55% of MEI results in RE and 42-47% is lost as heat (ME_m + activity + SDA) in plaice, *Pleuronectes platessa* (L.) and flounder when fed to satiation. These values are close to our determinations in turbot at feeding level I, but lower than at levels II and III. The higher proportions of RE in the present study are assumed to be attributed to the higher OC determined by Fonds et al. (1992) for plaice and flounder representing a higher energy loss by heat. The latter authors also compared the partitioning of MEI of flatfish with other fish and suggested that flatfish spend relatively less energy in heat and convert relatively more MEI to RE. They assumed the latter fact was caused by a reduced swimming activity of flatfish. Observations in our experiment will support this assumption as turbot spent most of their time lying on the bottom of the tanks.

4.4 Maintenance energy requirement and efficiency of energy utilization for growth

DE_m and ME_m of turbot in the present study (extrapolated from the linear regression) are in line with values reported in our previous experiments with turbot (17.0 and 15.5 kJ $kg^{-0.8}d^{-1}$ for DE_m and ME_m, respectively) and studies with other flatfish, but considerably lower than values for several common aquaculture species (Dietz et al., 2012). It has to be noted that in the latter experiment we used the comparative slaughter technique to determine E_N-loss, MEI, RE, DE_m and ME_m. Calculating E_N-loss in the present study by using data of our previous experiment is assumed to has less influence on the actual results, because E_N-loss contributes only a small proportion to the energy budget (3-6% of DEI and MEI; Kaushik, 1998b; Kaushik & Médale, 1994; Bureau et al., 2002).

Absent differences of DE_m or ME_m between salinities indicate that energetic costs for osmoregulation are quite small after adequate adaption. This is in agreement with the results of Tang et al. (2006) noted in the former sections. Because the determination of the energetic costs exclusively attributed to osmoregulation is difficult, there are no consistent data in the literature and values ranged from 10 to > 50% of the total energy budget (Boeuf et al., 2001). Additionally, Jobling (1994) noted that metabolic costs required for iono- and osmoregulation are considered to

be small and therefore any changes would be almost undetectable. Our results confirm this assumption. Despite there was no significant difference, DE_m and ME_m seemed to be reduced at a salinity of 30 g L^{-1} in the present study. According to the discussed aspects in the former sections we can only speculate at this point that the latter fact is explained by changes in spontaneous activity as well as the hormonal status rather than iono- and osmoregulation per se. The latter assumption is supported by Dutil et al. (1997), who showed higher growth rates in Atlantic cod at an intermediate salinity (14 g L^{-1}) and suggested that this was caused by factors like spontaneous activity rather than changes in standard metabolic rate.

Calculating ME_m directly from SOC (assumed to be close to standard metabolism) led to lower values (13.1 ± 2.2; 13.8 ± 1.4 and 11.1 ± 1.8 kJ $kg^{-0.8}d^{-1}$ for salinities of 10, 20 and 30 g L^{-1}, respectively) than determined by ROC. Regarding similar energetic costs for osmoregulation the latter differences may support the fact that starving fish showing less activity than fed fish (Jobling, 1994) and suggest a general effect of spontaneos activity on DE_m and ME_m in the present sudy. Furthermore, starving fish are also known to reduce their standard metabolic rate (Jobling, 1994) and therefore this may be another reason for the lower values in ME_m determined by SOC.

The k_g-values determined in the present study are considerably higher than in our previous experiment (0.592 and 0.632 for $k_{g\ (DE)}$ and $k_{g\ (ME)}$, respectively) as well as in studies with other flatfish and cultured fish species (Dietz et al., 2012). Just like in DE_m and ME_m no differences were determined in the k_g-values between salinities and lower DE_m and ME_m interrelated with reduced k_g-values. This correlation is in consistence with determinations in our previous experiment (Dietz et al., 2012) and may indicate reduced intermediary metabolic processes due to hormonal changes at 30 g L^{-1} salinity as discussed for OC, DE_m and ME_m. The similar RE between salinities in the present study resulted from lower DE_m- and ME_m-values compensated by lower k_g-values.

Referring to the total energy allocation the relative proportion of DE_m as well as ME_m decrease and of RE increase with increasing energy intake. Therefore, the k_g-values become more important than DE_m and ME_m when considering energy utilization and growth. Despite there were no differences in RE between salinities, we expect that turbot reared at a salinity of 20 g L^{-1} will show the highest energy utilization and growth at high energy intake due to the most favourable combination of DE_m and $k_{g\ (DE)}$ as well as for ME_m and $k_{g\ (ME)}$. This assumption is supported by results of Imsland et al. (2001), who showed best growth of juvenile turbot at salinities of 19-20 g L^{-1} and 18-22 °C. In this context, fish reared at a higher salinity (30 g L^{-1}) in the present study may show less energy utilization and growth, although had the lowest DE_m and ME_m. Morgan & Iwama (1991) suggest that optimal salinities for growth are also influenced by life history stage. Consequently, further growth experiments are needed, especially with larger fish, to confirm our latter assumptions.

The partial efficiencies of MEI utilization for protein and lipid retention in fish are reported to range between 0.44-0.56 and 0.72-0.91, respectively (Bureau et al., 2002; NRC, 2011). Consequently, it seems that the absolute values of the parameters determined in the present study are overestimated ($k_{g\ (DE)}$, $k_{g\ (ME)}$) and underestimated (ROC, SOC, DE_m, ME_m) to some extent. This was maybe in part a result of a drift of oxygen electrode calibration causing inaccurate dissolved oxygen measurements and respiration rate determinations (Eriksen, 2002). However, the latter aspects do not imply that the statistical results of the previous mentioned parameters between salinities are affected and therefore the general purpose of the present study as well.

Conclusion

Regarding the reduced nutrient and energy digestibility with increasing salinity in the present study it would be necessary to adjust the amount of supplied feed in turbot aquaculture dependent on salinity conditions.

Due to only small differences in SOC and ROC and similarities in DE_m and ME_m as well as $k_{g\ (DE)}$ and $k_{g\ (ME)}$ between different salinities in our experiment the energetic requirements for iono- and osmoregulation in juvenile turbot seems to be small in relation to the total energy budget. Consequently, turbot has good adaptability to salinity, which supports the euryhaline character of that species. We infer that respirometry accompanied with the determination of the N-balance can be suitable to determine estimates of DE_m and ME_m in fish, because of providing nearly similar results as the comparative slaughter technique.

It is concluded that at high energy intake turbot may show best energy utilization and growth at an intermediate salinity (20 g L^{-1}), whereas rearing them at a higher salinity (30 g L^{-1}) might decrease the latter parameters due to reduced k_g-values. On the other hand, our findings indicate that it is possible to expand the aquaculture of turbot to areas having lower salinities (10 g L^{-1}) without significant effects on energy utilization and growth. In this context, the influence of salinity on dietary digestibility has to be considered.

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General Discussion and Conclusion

Results of bioenergetic studies have been widely used for diet formulation and evaluation as well as to predict the energy balance of fish in aquaculture (Cho, 1992; Cho & Bureau, 1995; Lupatsch, 2009). The energy requirement of the fish is dependent on growth (potential, composition of weight gain) and demand for maintenance (Lupatsch, 2009). The challenges are to reduce feed costs, improve conversion efficiency and minimize environmental impact. One of the approaches is to accurately quantify energy and nutrient requirements of fish and choose feed ingredients according to least cost principle (Lupatsch, 2009). Considering an increasing turbot production (FAO, 2010, 2005-2012) decreasing market prices accompanied with lower profit margins are expected (Person Le-Ruyet, 2002). Nevertheless, there is no information about the maintenance energy requirements (DE_m, ME_m) and efficiencies of energy utilization for growth ($k_{g\ (DE)}$, $k_{g\ (ME)}$) available in the literature for that species. However, Fonds et al. (1992) indicated that flatfish having a lower energy expenditure (maintenance + activity + Specific dynamic action (SDA)) than other fish commonly kept in aquaculture. Therefore, the present experiments intended to determine DE_m and ME_m as well as $k_{g\ (DE)}$, $k_{g\ (ME)}$ using either a common growth trial in combination with the comparative slaughter technique (*chapter 1*) or respirometry (*chapter 2*). Furthermore, the effects of biotic (fish strain, dietary composition; *chapter 1*) and abiotic (salinity; *chapter 2*) factors on energy metabolism and energy budget of turbot were examined. In the respirometric experiment turbot of the same strain as well as a similar composed diet like in the growth trial were used to verify if both methods provide comparable results.

The results of both trials regarding the applied method and the effects of turbot strain, fish meal replacement by wheat gluten and salinity on dietary digestibility, energy budget and DE_m, ME_m, $k_{g\ (DE)}$, as well as $k_{g\ (ME)}$ are discussed in the following sections.

Methological comparison

Both methods (respirometry, comparative slaughter technique) to determine the energy balance in fish have their individual merits and limitations. The advantage of respirometry is that current observations on energy metabolism are possible. Small changes in energy balance and the effect of different experimental conditions (e.g. fish size; fish strain; temperature; salinity; dietary composition; nutritional status and history) can be studied within a relative short time period as well (Steffensen, 1989). Short time experiments also reduce problems of accumulation of products excreted by fish (Forstner, 1983). Additionally, it is not necessary to kill any fish and the same

individuum can be used for further measurements (Blaxter, 1967). On the other hand respirometry requires a complex technical equipment, an accurate measurement (i.e., correct calibration of probes) and careful consideration of several sources of errors, e.g., sustain a constant flow rate and an adequate recirculation of the water body, oxygen consumption by bacteria as well as appropriate ratios of fish size (Steffensen, 1989). Furthermore, short time periods of observation can differ from the real mean rate due to day-to-day variation, e.g., in activity or feed intake (Blaxter, 1967).

In contrast to fish respirometry, the comparative slaughter technique necessarily involves the slaughtering of fairly large groups of animals to reduce the inaccuracy in the estimation of RE (Blaxter, 1967). However, this method provide a direct measure of RE, limited only by analytical accuracy, space required, the necessity of long time intervals to ensure an adequate growth and by the basic assumption that initially reference and experimental groups of fish have the same energy content. With proper numbers of fish and randomization of individuals the latter assumption does not result in systematic error (Blaxter, 1967). Additionally, the sampled fish have to be representative for their respective reference or experimental group. The results of the comparative slaughter technique are in good agreement with those of indirect calorimetry (Bureau et al., 2002) and several studies have used this approach (Azevedo et al., 1998; Helland et al., 2010; Lupatsch et al., 2003; Rodehutscord & Pfeffer, 1999). Furthermore, the comparative slaughter technique can also be used to determine the retention and balance of macro- and micronutrients (protein, amino acids, lipid, vitamins, minerals).

DE_m and ME_m determined in the growth trial (17.0 and 15.5 kJ $kg^{-0.8}d^{-1}$, respectively) were close to values obtained by respirometry (14.9-19.1 and 13.3-17.3 kJ $kg^{-0.8}d^{-1}$, respectively), wheras $k_{g\ (DE)}$ and $k_{g\ (ME)}$ were considerably lower (0.59 and 0.63, respectively) when using the comparative slaughter technique than respirometry (0.82-0.86 and 0.86-0.91, respectively). This can maybe attributed to measuring inaccuracies regarding the oxygen consumption. Nevertheless, it was suggested that respirometry accompanied with the determination of the N-balance can provide results close to the comparative slaughter technique regarding the estimation of DE_m and ME_m. The latter is in agreement with the statements of Bureau et al. (2002).

Dietary digestibility

ADC-values in the current study based on the determinations at one feeding level only (1% BW). Commonly, most studies show that feeding levels have no impact on digestibility of nutrients (assuming that feed intake is not below levels needed to support reasonable growth rates) and intestinal absorptive capacity is not likely to be a limiting factor for nutrient digestibility (NRC,

2011). Therefore, the use of the same ADC-values of nutrients and energy for all feeding levels in the present study is considered to be acceptable.

Due to low amounts of digesta sampled in the hind gut, it was impossible to realize chemical analyses within each replicate. Thus, we decided to pool the samples from all replicates within each diet (growth trial) and salinity (respirometric trial). Therefore, statistical analysis was not possible and consequently resulting in estimated ADC-values. However, the observed ADC-values can be considered as representative as ADC-values of dietary nutrients and energy are in the range of values determined by other studies with turbot (Fournier et al., 2004; Oliva-Teles et al., 1999; Peres & Oliva-Teles, 2005; Regost et al., 2001, 2003) and Atlantic halibut (*Hippoglossus hippoglossus*) (Berge & Storebakken, 1991; Grisdale-Helland & Helland, 1998) at similar dietary composition. Variations of ADC-values between studies may be caused by using different feces collecting methods.

Due to pooling fish from DK and IS related to the ADC determination in the growth trial the effect of fish strain on ADC of nutrients and energy could not be investigated. Furthermore, it appears that ADC-values of energy and lipid were not affected when replacing fish meal by wheat gluten, whereas the ADC of protein seems to slightly increase. The improved ADC of protein maybe attributed to a higher protein digestibility of wheat gluten than fish meal potentially including less digestible proteins from bones or scales (NRC, 2011). ADC-values of energy and protein seemed to decrease at increasing salinities according to several studies with other fish species (Ferraris et al., 1986; MacLeod, 1977; Ringø, 1991; Storebakken et al., 1998; Woo & Kelly, 1995). This reduction can likely attributed to higher drinking rates at increasing salinities (Boeuf & Payan, 2001), which could consequently increase gastric emptying rate, intestinal passage rate and pH in the digestive tract as well as decrease feed breakdown and absorption of nutrients and energy (Ferraris et al., 1986; MacLeod, 1977; Usher et al., 1990).

Energy budget

Energy losses by feces in the present experiments were in the range (15-40% of GE) commonly reported for fish fed commercial diets (Cho & Bureau, 1995; NRC, 2011). Fecal energy loss was lower in the respirometric experiment (13-16% of GE) than in the growth trial (21% of GE) probably due to the use of different fish sizes, which is known to potentially influence nutrient and energy digestibility, with small fish seems to digest feed less efficiently than larger fish (Jobling, 1994; Ferraris et al., 1986). N-excretion is directly correlated with DPI (Ballestrazzi et al., 1994; Beamish & Thomas, 1984; Dosdat et al., 1995; Kaushik & Medale, 1994) and in consequence with

energy intake from protein (DEI_p) as well. The latter was confirmed in the present study as the replacement of fish meal by wheat gluten increase DEI_p accompanied with slightly higher non-fecal energy losses likely a result of the slightly increased dietary protein content and ADC of protein. Despite an apparent increase of energy losses by N-excretion with increasing salinity (due to the influence of salinity on the ADC of protein) no significant difference in N-excretion between salinities was observed in general. The average non-fecal energy loss in both studies ranged between 6-7% of DE intake and was similar to commonly denoted values of 3-6% (Bureau et al., 2002; Kaushik & Médale, 1994).

Fonds et al. (1992) showed that 53-55% of ME intake results in RE and 42-47% is lost as heat (ME_m + activity + SDA) in plaice (*Pleuronectes platessa*) and flounder (*Platichthys flesus*) when fed to satiation. The latter proportion of RE is similar to results at the highest (1.2-1.3% of BW), but lowest (0.3% of BW) feed intake in the growth and respirometric trial, respectively. The higher proportion of RE at the higher feed intake levels (0.6-0.9% of BW) in the respirometric trial compared to the study of Fonds et al. (1992) may due to the higher oxygen consumption recorded for plaice and flounder representing a higher energy loss by heat. The proportion of retained energy as protein of RE was unaffected by turbot strain and the replacement of fish meal by wheat gluten, thus, confirming results of Helland & Grisdale-Helland (2006), who observed also no effect of replacing fish meal by wheat gluten in diets for Atlantic halibut (*Hippoglossus hippoglossus* L.) on protein as well as energy retention. The experiments in the present study showed that neither the strain of turbot, a replacement of fish meal by wheat gluten nor ambient salinity affected total RE.

Maintenance energy requirement and efficiency of energy utilization for growth

DE_m and ME_m are known to be influenced by several factors, such as BW, temperature, fish species and diet composition (Azevedo et al., 1998; Bureau et al., 2002; Jobling, 1994; Lupatsch et al., 2003). The results of the growth trial showed differences in DE_m and ME_m between the two strains of turbot and support the observations of Imsland & Jonassen (2001), who indicated that energy requirements (maintenance + activity + SDA) can differ between distinct strains of Atlantic halibut as well. $K_{g\ (DE)}$ and $k_{g\ (ME)}$ in the present study also differed between the two strains of turbot and being at the lower point of the range of values determined in other aquaculture species like European sea bass (*Dicentrarchus labrax*), gilthead sea bream (*Sparus aurata*), white grouper (*Epinephelus aeneus*) (Lupatsch et al., 2003) and barramundi (*Lates calcarifer*) (Glencross, 2008). Therefore, results of the present study support the statement of Lupatsch (2009) that maintenance requirement expressed per kg metabolic body weight is species specific, but contradict the position

that k_g is relatively constant and independent from fish species. Although not directly comparable to the present results Imsland et al. (2000) observed differences in $k_{g\ (GE)}$ in various populations of Atlantic halibut, which suggests that intraspecific variations maybe also generally present in turbot considering $k_{g\ (DE)}$ and $k_{g\ (ME)}$. Additionally, Jobling (1994) noted that breeding as well as hatchery programes in aquaculture usually aim in production of fast growing strains of fish. The latter author pointed out that strains known to grow most rapidly and most efficiently often show low rates of protein breakdown and turnover leading to lower costs to maintain status quo. Therefore, it is hypothesized that the differences in DE_m and ME_m as well as $k_{g\ (DE)}$ and $k_{g\ (ME)}$ between strains in the present study may reflect different periods of breeding and hatchery selection. However, detailed informations on time of domestication and the genetic background of both strains of turbot in the present study were not available.

Whereas no reference was available for fish indicating effects of dietary protein source on the maintenance energy requirement, Nieto et al. (1995) showed that feeding a diet deficient in one indispensable amino acid to growing chickens increase ME_m and $k_{g\ (ME)}$. This effect was assumed to be caused by an intensified muscle protein breakdown resulting in higher energetic costs for protein turnover as well as a decreased and increased efficiency of protein and lipid retention, respectively. Replacing dietary fish meal by wheat gluten seems to cause a similar effect than observed by the latter authors, however, in diets not deficient in any indispensable amino acid. Whether the results in the present study were also caused by changes in body protein breakdown needs to be further investigated. Overall, results in the present study show that DE_m of turbot was lower than reported for most other aquaculture species and support the suggestion of Fonds et al. (1992) mentioned in the former sections.

It was shown that DE_m and ME_m as well as $k_{g\ (DE)}$ and $k_{g\ (ME)}$ were similar at different salinities and being in consistence with observations by Tang et al. (2006), who showed similar metabolic rates after 48 h of adaption to changed salinities. Thus, results of the present study support the statement that metabolic costs required for iono- and osmoregulation are small and therefore any changes would be almost undetectable (Jobling, 1994). Despite the lack of significant differences, it seemed that DE_m and ME_m as well as $k_{g\ (DE)}$ and $k_{g\ (ME)}$ decreased at higher salinities (30 g L^{-1}). Several studies (Boeuf et al., 1999; Boeuf & Payan, 2001; Morgan & Iwama, 2001) mentioned the role of hormonal changes (e.g. cortisol, growth hormone, thyroid hormones), also known to affect metabolic rate and growth of fish, related to changing salinities. Therefore, the lower values of DE_m and ME_m as well as $k_{g\ (DE)}$ and $k_{g\ (ME)}$ determined at a salinity of 30 g L^{-1} were probably caused by changes in hormonal status or spontaneous activity (Dutil et al., 1997) as well, rather than osmoregulation per se.

Referring to the total energy allocation the relative proportion of DE_m as well as ME_m decrease and of RE increase with increasing energy intake. Therefore, the k_g-values become more important than DE_m and ME_m when considering energy utilization and growth. In this context turbot reared at an intermediate salinity (20 g L^{-1}) showed the most favourable combination of DE_m and $k_{g\ (DE)}$ as well as for ME_m and $k_{g\ (ME)}$ for highest energy utilization and growth at high energy intake. This assumption is supported by results of Imsland et al. (2001b), who showed best growth of juvenile turbot at a salinity of 19-20 g L^{-1}.

Practical implications

In total, results of the present study provide basic data of the energy metabolism of juvenile turbot which may allow reasonably accurate feeding standards to be calculated for aquaculture production of that species. The latter can reduce feed costs and therefore may ensure an adequate profit margin. Furthermore, the present study implies that different strains of turbot having different characters regarding the energy metabolism, which indicates that it might be advisable to consider the strain of fish when selecting turbot used in aquaculture related to production purposes. However, this suggestion has to be verified in further experiments, especially with larger fish, as no significant differences in RE have been observed between strains. Additionally, further research is needed to evaluate if it would be necessary to adjust the energy supply when fish meal is replaced by wheat gluten or other protein sources due to changes in energy metabolism. Nevertheless, wheat gluten can be used up to 33% in turbot diets without reducing RE and growth.

Results in the present study suggest that rearing juvenile turbot at an intermediate salinity (20 g L^{-1}) will provide the prerequisites for highest energy utilization and growth at high energy intake. Furthermore, rearing juvenile turbot at a lower salinity (10 g L^{-1}) caused no significant effect on the latter parameters. As cage culture of turbot is considered to be in its pilot stage and location a crucial factor regarding productivity (FAO, 2005-2012), the present results indicate the opportunity to expand turbot aquaculture to areas with lower salinities. In this context, the influence of salinity on dietary digestibility has to be considered. Consequently, energy and nutrient supply should increase with elevated salinity levels.

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Summary

Aquaculture continues to be the fastest-growing branch producing foods from animal origin. In Europe the production of turbot (*Psetta maxima*), a species of high commercial value, rapidly developed over the last decade and is highest among flatfishes. Considering an expected increase in turbot production accompanied with decreasing market prices it is of highest relevance to improve the production efficiency of turbot farming to maintain an adequate profit margin. Feeding efficiency is a crucial factor, because feeds represent a minimum of 17% of the total production costs. In addition to the energy required for maintenance the feed must supply the precursors for new tissue production as well as the energy necessary for synthesis of both protein and lipid. Informations about the maintenance energy requirement and the efficiency of energy utilization for growth are the basis for a performance-related feeding and a corresponding diet formulation.

In the present study the digestible and metabolizable energy requirements for maintenance (DE_m; ME_m) as well as the respective efficiencies of energy utilization for growth ($k_{g\ (DE)}$; $k_{g\ (ME)}$) in juvenile turbot were determined and the effects of biotic or abiotic factors examined. Two different methods for determination were applied.

In *chapter 1* growth experiments accompanied with comparative whole body composition analyses were realized to determine DE_m and ME_m as well as $k_{g\ (DE)}$ and $k_{g\ (ME)}$. The effects of the fish strain (Denmark, DK; Iceland, IS) as well as the partially replacement of fish meal by wheat gluten in the diet were examined as possible factors affecting the energy metabolism. Turbot were reared in a recirculation aquaculture system for 67 days. ME_m and DE_m were 15.5-21.4 and 17.0-23.5 kJ $kg^{-0.8}$ d^{-1}, respectively, and $k_{g\ (ME)}$ and $k_{g\ (DE)}$ were 0.63-0.68 and 0.59-0.64, respectively. ME_m and DE_m as well as for $k_{g\ (ME)}$ and $k_{g\ (DE)}$ in turbot from IS were higher than in fish from DK, but without any effect on energy retention. Turbot receiving the diet where fish meal was partially replaced by wheat gluten showed higher DE_m but not ME_m. No effect on energy retention was observed.

In *chapter 2* oxygen consumption (OC) was measured using flow-through respirometry to determine DE_m and ME_m as well as $k_{g\ (DE)}$ and $k_{g\ (ME)}$ and to examine the influence of salinity on energy metabolism in juvenile turbot. Turbot were reared in a recirculation respirometry system at three different salinities (10, 20, 30 g L^{-1}) and OC was recorded for a 24 h period. There is evidence that nutrient and energy digestibilities decreased with increasing salinity. DE_m and ME_m, $k_{g\ (DE)}$ and $k_{g\ (ME)}$ were 14.9-20.2 and 13.3-18.3 kJ $kg^{-0.8}d^{-1}$, 0.82-0.87 and 0.87-0.91, respectively. Generally, no differences were observed in DE_m, ME_m, $k_{g\ (DE)}$, and $k_{g\ (ME)}$ between salinities, but it was indicated that these parameters decrease at a salinity of 30 g L^{-1}. Furthermore, turbot showed the most favourable combination of DE_m and $k_{g\ (DE)}$ as well as ME_m and $k_{g\ (ME)}$ at salinity of 20 g L^{-1}

concerning growth and energy utilization at high energy intake, whereas these combinations became less favourable at 30 g L^{-1}.

Results from experiments in the present study show that both methods are equally suitable to determine DE_m and ME_m in juvenile turbot. Differences in energy metabolism between various strains might be considered when selecting turbot for aquaculture production. It was shown that the partial replacement of fish meal by wheat gluten increased DE_m and $k_{g\ (DE)}$ as well as ME_m and $k_{g\ (ME)}$. Nevertheless, wheat gluten can replace fish meal up to a total inclusion level of 330 g kg^{-1} in diets for juvenile turbot without negative effects on growth performance and energy retention. Furthermore, the present study show that the energetic requirements for iono- and osmoregulation in juvenile turbot are small and fish have the most favourable attributes for high energy utilization at an intermediate salinity (20 g L^{-1}), whereas these attributes become slightly worse at a higher salinity, which maybe have adverse implications for aquaculture production. The results of the present study indicate that it is possible to expand the aquaculture of turbot to areas having lower salinities (10 g L^{-1}). The influence of salinity on nutrient and energy digestibility seems to be an important factor to be considered in this context.

Zusammenfassung

Aquakultur stellt weiterhin den am schnellsten wachsenden Produktionszweig bei der Erzeugung tierischer Lebensmittel dar. In Europa hat sich innerhalb des letzten Jahrzehnts die Kultivierung von Steinbutt (*Psetta maxima*), einer Art von hohem wirtschaftlichem Wert, stark entwickelt und ist gegenwärtig am höchsten unter den Plattfischarten. In Anbetracht einer voraussichtlich weiter ansteigenden Produktion einhergehend mit sinkenden Marktpreisen, ist die Verbesserung der Wirtschaftlichkeit der Steinbutthaltung entscheidend, um eine angemessene Gewinnspanne aufrecht zu erhalten. Die Futterverwertung ist hierbei ein wesentlicher Faktor, da Futterkosten zu mindestens 17% der gesamten Produktionsausgaben beitragen. Zusätzlich zur Energie für den Erhaltungsbedarf muss das Futter die zum Aufbau neuer Körpersubstanz notwendigen Ausgangsstoffe liefern sowie die dafür nötige Energie zur Protein- und Fettsynthese bereitstellen. Informationen zum Energieerhaltungsbedarf und zur Effizienz der Energieverwertung für das Wachstum sind die Basis für eine leistungsgerechte Fütterung und eine entsprechende Rationsgestaltung.

In der vorliegenden Dissertation wurden der Erhaltungsbedarf für verdauliche und umsetzbare Energie (DE_m; ME_m) sowie die jeweiligen Effizienzen der Energieverwertung für das Wachstum ($k_{g\ (DE)}$; $k_{g\ (ME)}$) beim juvenilen Steinbutt bestimmt und der Einfluss von biotischen und abiotischen Faktoren untersucht. Zwei methodisch unterschiedliche Herangehensweisen wurden dabei gewählt.

In *Kapitel 1* wurden Wachstumsversuche in Verbindung mit Ganzkörperanalysen durchgeführt, um DE_m und ME_m sowie $k_{g\ (DE)}$ und $k_{g\ (ME)}$ zu bestimmen. Dabei wurden die Effekte von Fischstamm (Dänemark, DK; Island, IS) sowie des partiellen Ersatzes von Fischmehl durch Weizengluten im Futter als potentielle Einflussgrößen auf den Energiestoffwechsel untersucht. Die Steinbutte wurden 67 Tage in einer Aquakultur-Kreislaufanlage gehalten. ME_m und DE_m betrugen 15,5-21,4 beziehungsweise 17,0-23,5 kJ $kg^{-0,8}d^{-1}$ und $k_{g\ (ME)}$ sowie $k_{g\ (DE)}$ 0,63-0,68 beziehungsweise 0,59-0,64. ME_m und DE_m als auch $k_{g\ (ME)}$ und $k_{g\ (DE)}$ lagen bei den Steinbutten aus IS höher als bei denen aus DK, ohne aber die Energieretention nennenswert zu beeinflussen. Steinbutte die Rationen, bei denen Fischmehl teilweise durch Weizengluten ausgetauscht wurde, erhielten, wiesen einen höheren DE_m nicht aber ME_m auf. Ein Effekt auf die Energieretention wurde nicht beobachtet.

In *Kapitel 2* wurde der Sauerstoffverbrauch (OC) mittels Durchfluss-Respirometrie gemessen, um DE_m und ME_m sowie $k_{g\ (DE)}$ und $k_{g\ (ME)}$ zu bestimmen und den Einfluss des Salzgehaltes auf den Energiestoffwechsel beim juvenilen Steinbutt zu untersuchen. Die Steinbutte wurden dafür in einem als Kreislaufanlage konzipierten Respirometersystem bei drei unterschiedlichen Salzkonzentrationen (10, 20, 30 g L^{-1}) gehalten und der OC für einen Zeitraum von 24 Stunden aufgezeichnet.

Es deutete sich an, dass die Nährstoff- und Energieverdaulichkeit mit ansteigendem Salzgehalt abnimmt. DE_m und ME_m sowie $k_{g\ (DE)}$ und $k_{g\ (ME)}$ betrugen jeweils 14,9-20,2 und 13,3-18,3 kJ $kg^{-0,8}d^{-1}$ sowie 0,82-0,87 und 0,87-0,91. Grundsätzlich konnten keine Unterschiede bei DE_m, ME_m, $k_{g\ (DE)}$, und $k_{g\ (ME)}$ zwischen den Salzkonzentrationen festgestellt werden. Es zeichnet sich jedoch ab, dass diese Kenngrößen bei einem Salzgehalt von 30 g L^{-1} abnehmen. Außerdem wiesen die Steinbutte bei einem Salzgehalt von 20 g L^{-1} die günstigste Kombination von DE_m und $k_{g\ (DE)}$ beziehungsweise ME_m und $k_{g\ (ME)}$ für das Wachstum und die Energieverwertung bei hoher Energieaufnahme auf, wohingegen diese bei 30 g L^{-1} etwas ungünstiger ausfielen.

Die Ergebnisse aus den Versuchen der vorliegenden Arbeit zeigen, dass beide Methoden gleichermaßen geeignet sind DE_m und ME_m beim juvenilen Steinbutt zu bestimmen. Die Unterschiede im Energiestoffwechsel zwischen verschiedenen Stämmen sind bei der Auswahl von Steinbutten für die Aquakultur möglicherweise zu berücksichtigen. Es konnte gezeigt werden, dass sich DE_m und $k_{g\ (DE)}$ sowie ME_m und $k_{g\ (ME)}$ durch den partiellen Austausch von Fischmehl gegen Weizengluten erhöhen. Dennoch kann Weizengluten als Fischmehlsubstitut bis zu einem Anteil von 330 g kg^{-1} im Futter für juvenile Steinbutte eingesetzt werden, ohne sich negativ auf die Wachstumsleistung und die Energieretention auszuwirken. Weiterhin zeigt die vorliegende Arbeit, dass der Energiebedarf für Ionen- und Osmoregulation beim juvenilen Steinbutt nur gering ist und die Fische bei mittleren Salzkonzentrationen (20 g L^{-1}) die günstigsten Voraussetzungen für eine hohe Energieverwertung aufweisen, wohingegen diese sich bei höherem Salzgehalt geringfügig verschlechtern, was sich nachteilig auf die Produktion auswirken könnte. Demgegenüber deutet die aktuelle Studie an, dass es möglich ist die Aquakultur von Steinbutt auch auf Gebiete mit niedrigerem Salzgehalt (10 g L^{-1}) auszudehnen. Der Einfluss des Salzgehaltes auf die Nährstoff- und Energieverdaulichkeit scheint ein wesentlicher Faktor zu sein, der in diesem Zusammenhang zu beachten ist.

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